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IMMUNOGLOBULIN E AND THE MAST CELL
The Rat as an Animal Model in Allergy Research

by

TORBJÖRN KARLSSON

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To Mons, Elias and

the Hooded Lister Rat

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IMMUNOGLOBULIN E AND THE MAST CELL

The Rat as an Animal Model in Allergy Research

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Torbjörn Karlsson

Fil. kand., Skånel.

UPPSALA 1978

SUMMARY

This thesis presents a new system for the quantitative measurement of total IgE (PRIST) and IgE antibody (PRAST) in rat serum. The radioimmunoassays are based on paper disc coupled anti-IgE antibodies and antigen, with ^{125}I anti Fc-ε used as an indicator of IgE uptake, and allow the measurement of total IgE and IgE antibody below 1 ng per ml and are thus more sensitive than other rat IgE assays described to date. PRAST is the first in vitro assay that has been used for rat IgE antibody. PRAST was also adopted for evaluation of the relative amount of IgG antibody produced in immunization experiments. Differences between rat strains in normal serum IgE levels and in IgE antibody production after parasitic infection and immunization are shown. Hybridization experiments indicate a poly-gene control of the serum IgE level. Experiments with isolated rat mast cells show differences between a high IgE strain (Hooded Lister) and a low IgE strain (Sprague Dawley) in the number of occupied IgE receptors. The number of IgE receptors per mast cell is independent of the serum IgE level of the strain. Histamine release experiments with isolated mast cells, nonsensitized and passively sensitized, show a better immune reactivity of cells from the high IgE than the low IgE strain. Overly high serum IgE levels are shown to interfere with the use of these rats for IgE antibody determination by passive cutaneous anaphylaxis. An attempt to quantitate the amount of IgE antibody necessary to give a positive PCA response is also made. External labelling of cells from the immune system show unique proteins on the surface of mast cells and other peritoneal cells.

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I. INTRODUCTION

Reagins, which play a central role in immediate type hypersensitivity reactions, belong to a distinct class of serum immunoglobulin (Ig) denoted IgE (Ishizaka and Ishizaka, 1967; Johansson and Bennich, 1967a). The discovery of human IgE myeloma proteins (Johansson and Bennich, 1967b) has greatly advanced the development of radioimmunological assays for serum IgE (Johansson et al., 1968a) and for the in vitro diagnosis of allergy (Wide et al., 1967) and, furthermore, has provided the means for studies of the conformational properties (Dorrington and Bennich, 1978) and covalent structure of the IgE molecule (Bennich et al., 1978).

The mediator function of IgE antibody in allergic reactions is explained by its unique ability to bind tightly but reversibly to membrane receptors on blood basophilic leukocytes and tissue mast cells (Ishizaka et al., 1970a) via receptor sites located in the Fc portion of the molecule (Stanworth et al., 1968). A reaction between such membrane bound IgE antibody and a multivalent antigen (allergen), by an unknown mechanism, induces a series intracellular reactions, which lead to the release of histamine and other vasoactive mediators. The IgE receptor on the mast cell membrane has not been characterized in detail. Recent studies indicate that it may be a glycoprotein (Kulczycki et al., 1976) with a subunit molecular weight of 62,000-77,000 daltons (Conrad and Froese, 1976; Newman et al., 1977) and a functional unovalence (Newman et al., 1977).

Interest in the IgE-mast cell system has mainly focused on its role in allergic reactions, an understandable emphasis, in view of the negative effects and the high frequences of allergic diseases. There are, however, reasons to believe that the IgE-mast cell system has a normal function of definite survival value, as the system is to be found in most vertebrate species.

To elucidate what function IgE may play in relation to other classes of humoral antibody and to determine the possible role of the mast cell in relation to the functions of T and B lymphocytes for the development of a normal immune response, an animal model system is required which permits not only qualitative but also quantitative studies at the cellular and molecular levels. Until recently, man has remained unique to students of IgE as being the single species in which IgE secreting plasma cell tumours are known to occur. The discovery of IgE producing plasma cell tumours in the LOU strain of rats by Bazin and co-workers (1974) therefore represents an important achievement as it provides the means that were previously missing in experimental studies of the biologic functions of IgE.

In view of the low frequency of human IgE myelomas (not more than 12 cases have been described since 1967) the finding that more than 40 per cent of spontaneously occurring plasmacytomas in rats of the LOU/C strain are secreting IgE is indeed remarkable. The general characteristics of these and other classes of immunocytomas have recently been reviewed by Bazin and Beckers (1976). IgE producing tumours can be transplanted into syngenic hosts by either subcutaneous or intraperitoneal injection resulting in serum (or ascitic) levels of IgE in the range of 5-35 mg per ml after 2-3 weeks. Although a great number of the immunocytomas seemed to lose their ability to synthetize immunoglobulin after a few passages in vivo, at least 4 tumour lines were found which have retained their original ability to synthetize and secrete monoclonal IgE for more than 2 years (Bazin and Becker, 1976). Two of these supposedly stable tumour lines - IR2 and IR162 - were kindly put at our disposal by Dr. Bazin and have been maintained since 1975 by serial passage in LOU rats.

Until rat IgE myeloma proteins became available, thus allowing the preparation of specific antisera, no means were at hand for quantitative measurement of the IgE levels in nonimmunized rats. The concentration of IgE is usually too low even in serum from

immunized animals to be measured by classic procedures such as single radial immunodiffusion (Mancini et al., 1965) and therefore, radioimmunoassays or methods having an equivalent or greater sensitivity are required. Jarrett and Bazin (1974) using the radioactive single diffusion technique of Rowe (1969) were the first to demonstrate that helminth infection of rats will lead to the production of very high levels of total serum IgE in both immunized (with a nonparasite antigen) and nonimmunized animals and, furthermore, that the magnitude of such a potentiating effect is strain dependent. As the radioactive Mancini technique is not very accurate in the low ranges, the IgE levels in normal rats below 0.35 µg per ml could not be measured. A radioimmunoassay for the measurement of total IgE using ammonium sulphate precipitation has been described by Carson et al. (1975a). The assay, which is based on a difference in solubility of free and antibody bound IgE in 33 per cent saturated ammonium sulphate, was utilized in a subsequent study (Carson et al., 1975b) to follow the changes in the IgE level during the potentiated reagin response to ovalbumin in Sprague Dawley rats infected with Nippostrongylus brasiliensis. The results of this study were in agreement with those obtained by parasitic infection of Hooded Lister and Wistar rats as previously reported by Jarrett and Bazin (1974). In a similar study by Ishizaka et al.(1976), total serum IgE levels in infected animals were measured by using a radioimmunoassay based on a double antibody technique. However, in none of the above studies was the sensitivity of the assays utilized to evaluate either fluctuations in the basic total IgE levels prior to infection with parasite or immunization with antigen or to determine the serum IgE levels during the early immune response. For determination of IgE antibody in the serum of infected and/or immunized rats the procedure of passive cutaneous anaphylaxis (PCA), originally described by Ovary (1952), has been used by most investigators.

Rat myeloma IgE, although devoid of any known antibody activity, has retained most other biological properties of the rat reaginic antibody. Purified monoclonal IgE has the ability to inhibit the

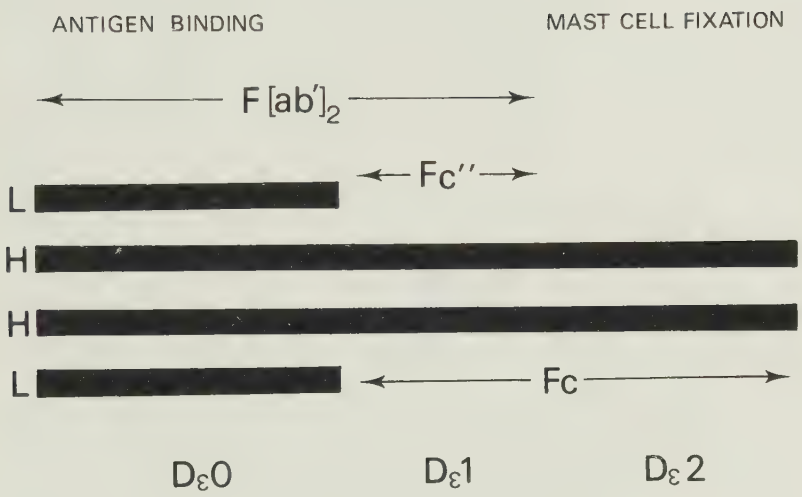


Fig. 1. Diagrammatic structure of IgE illustrating the molecular location of the enzymic fragments $F(ab')_2$-ε, Fc-ε and Fc"-ε and the distribution of antigenic determinant groups (D_ε). (Modified from Bennich and von Bahr-Lindström, 1974).

PCA reaction when added to reaginic serum, which, together with the fact that reaginic serum cannot induce a positive PCA reaction in LOU rats carrying IgE secreting tumours, indicates that rat myeloma IgE competes with reaginic antibody for the same receptor sites present on the mast cell. The finding that antisera raised against the purified myeloma protein will remove all reaginic activity in a PCA positive serum, and also has the capacity to induce cutaneous anaphylactic reactions of the reversed type in the skin of a PCA positive recipient, indicates that monoclonal IgE and reaginic antibody share major class specific antigenic determinants. The physicochemical properties of rat IgE have been found to be very similar to those of human IgE. The average molecular size of three E myeloma proteins has been estimated to be 180,000 ± 8,000, as determined by SDS-polyacrylamide gel electrophoresis, and 179,000 by ultracentrifugation analysis. The molecular size of the epsilon heavy chain was estimated to be 68,000 by SDS-polyacrylamide gel electrophoresis (Bazin and Beckers, 1976). Although, neither the antigenic nor covalent structure of rat IgE is known in detail as yet, there are good reasons to believe that the structure of human IgE, as shown diagrammatically in Fig.1, can also be applied to rat IgE.

Aim of present investigation

1. To design a procedure for radioimmunoassay of total serum IgE in the rat allowing measurements at the nanogram level.

2. To design a sensitive radioimmunoassay to replace PCA for determination of the IgE antibody response after immunization and/or parasitic infection of the rat.

3. To evaluate, by using the above methods, any differences in production of total IgE and IgE antibody between different strains of rats.

4. To study the interaction of IgE and mast cells.

II. MATERIALS

1. Adjuvants

Aluminium hydroxide gel for immunization was prepared as described by Levine and Vaz (1970). Freund's complete (FCA) and incomplete (FIA) adjuvant were purchased from Difco Laboratories, Detroit, Michigan, USA. Bordetella pertussis vaccine (2×10^{10} bacteria per ml) was purchased from Statens Bakteriologiska Laboratorium, Stockholm, Sweden.

2. Animals

New Zealand White rabbits for production of antisera were purchased from Försvarets Fabriksverk, Åkers Styckebruk, Sweden. The rats used in the present study were from colonies maintained under controlled environmental conditions in the Animal Department at The Biomedical Center in Uppsala and originated from parental strains supplied by Anticimex AB, Märsta, Sweden (Sprague Dawley), Dr. H. Bazin, University of Louvain, Brussels, Belgium (LOU/C and LOU/M) and Møllegaard Avlslaboratorium ApS, L. Skensved, Denmark (Hooded Lister). Rats and rabbits were fed with commercially available pellets (Astra-Ewos AB, Södertälje, Sweden) and given tap water ad libitum.

3. Antigens

Dog albumin (A-9263, Fraction V) and ovalbumin (Chicken egg, A-5503, grade V) were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. Nippostrongylus brasiliensis antigen, extracted from adult worms (Ogilvie, 1967), was generously supplied by Dr. Ellen Jarrett, The Wellcome Laboratories for Experimental Pathology, University of Glasgow, Scotland. Rabbit IgG was isolated from pooled normal rabbit (New Zealand White) serum by ammonium sulphate precipitation (40 per cent SAS), ion exchange chromatography and gel filtration. Monoclonal rat IgE and rat IgM and normal rat IgG were purified as described in the method section.

4. Antisera

Antisera to rat IgE(IR162), rat IgM(IR202) and normal rat IgG were prepared in rabbits by using the following protocol:

Equal volumes of antigen dissolved in PBS (1 mg per ml) and FCA were thoroughly mixed immediately prior to administration. Each rabbit received 2 x 0.5 ml of emulsified antigen injected i.m. in the hind legs on day 0, 14 and 28. Two weeks after the second injection, blood was collected from the ear vein for determination of the antibody titer. One week later, about 50 ml blood were collected from animals that had a serum antibody titer of at least 1 mg per ml. Seven weeks after the first injection, the animals were boosted with the same amount of antigen as given initially, but emulsified in FIA. Two weeks later, the animals were bled, the blood was allowed to clot at RT and serum was collected by centrifugation (10,000 x g, 30 min, 6-8^{o}C). All antisera were kept frozen at -30^{o}C until further processed. Antibody to purified Hooded Lister rat mast cells (native or treated by exposure to pH 3 buffer) was prepared in rabbits using the protocol as described above. 1-2 x 10^{7} cells in 0.5 ml PBS and emulsified in an equal volume of FCA were injected i.m. Antibody to normal rabbit IgG was prepared in the goat using the protocol described above. Goat antiserum to rat IgE(IR2) and rat IgM(IR202) were kindly supplied by Dr. H. Bazin, University of Louvain, Brussels, Belgium. Rabbit antiserum to normal rat IgG and normal rat serum were purchased from Pentex (Miles Research Laboratories, Inc., Kanakee, Illinois, USA).

5. Buffers

Compositions given below refer to the concentration of salt etc. in one liter of buffer.

'Assay' buffer for PRIST and PRAST (Ceska and Lundkvist, 1972). 46.5 mM Na_2HPO_4; 3.5 mM KH_2PO_4; 150 mM NaCl; 1 g Bovine serum albumin (Fraction V) - Miles Laboratories Ltd., Slough, England; 1.0 ml Tween 20 (polyoxyethylene sorbitan-monolaurate) - Sigma Chemical Company, St. Louis, USA; 0.5 g NaN_3. pH = 7.9.

MCM-3 buffer, for dissociation of mast cell membrane bound IgE. (Ishizaka and Ishizaka, 1974). 50 mM DL-glycine; 85 mM NaCl; 5 mM KCl; 10 mM disodium-EDTA; 16.4 mM HCl; 0.3 g Bovine serum albumin (fraction V). pH = 3.0.

MCM-7 buffer, for histamine release (Sullivan et al., 1975b)
150 mM NaCl; 3.7 mM KCl; 3.0 mM Na_2PO_4; 3.5 mM KH_2PO_4;
0.9 mM $CaCl_2$; 5.6 mM D-glucose; 1.0 g Bovine serum albumin,
1.6 g Gelatin (Bacto)-Difco Laboratories, Detroit, Mich., USA.
pH = 6.8.

NP40-PBS, for dissociation of membrane components.
Phosphate buffer saline (PBS) containing:
10 ml Nonidet P40 (NP40) - BDH Chemicals Ltd., Poole, England;
3.5 mM Phenylmethyl sulphonyl fluoride (PMSF) - Sigma Chemical
Company, St. Louis, USA; 3.5 mM Iodoacetamide, recrystallized;
7 g Trasylol - Bayer AG, Leverkusen, W-Germany. pH = 7.2.

Phophate buffer saline (PBS) (Dulbecco and Vogt, 1954).
Dulbecco's phosphate buffer saline without calcium or magnesium.
pH = 7.2.

SDS-Fairbanks buffer, for polyacrylamide gel electrophoresis (PAGE),
(Fairbanks et al., 1971).
Stock solution (concentrated) without sodium dodecyl sulphate (SDS):
0.40 M Trizma Base - Sigma, 0.20 M sodium acetate; 20 mM disodium-
EDTA; 0.25 M acetic acid. pH = 7.4.

For SDS-PAGE and SDS-gel filtration analysis:
10 times diluted stock solution containing 0.2 per cent SDS.

TBS-7.7, for affinity and gel chromatography.
0.1 M TRIZMA Base - Sigma; 0.08 M HCl; 0.2 M NaCl; 2 mM disodium
EDTA; 0.2 g NaN_3. pH = 7.7.

6. Cyanogen bromide activated paper discs (Ceska and Lundkvist, 1972).
Paper discs, 6 mm diameter, were prepared from 47 x 47 cm sheets of
cellulose filter paper (Munktell OOH-S1-80-40, purchased from
Grycksbo Pappersbruk AB, Grycksbo, Sweden), by using a punch. To
20 grams of paper discs (about 10,000), soaked in 200 ml of distilled
water for 10 mins at RT, were added 600 ml of a freshly prepared

solution of cyanogen bromide (20 grams of CNBr per 600 ml distilled water). The pH was adjusted to 10.5(± 0.5) by addition of freshly prepared 1 M sodium hydroxide. When 100 ml of base were consumed, the reaction was interrupted by addition of crushed ice. The discs were washed 10 times in 1 L of ice-cold 5 mM sodium bicarbonate, pH 9; twice in 1 L of distilled water, twice in 0.5 L of each of the following concentrations of acetone: 25, 50 and 75 per cent followed by 4 washings in 0.5 L absolute acetone. Excess acetone was removed by leaving the discs on a large sheet of filter paper for about 1 hr at RT. Finally, the activated paper discs were transferred to a desiccator, where they were kept under vacuum over P_2O_5 until further processed.

7. Cyanogen bromide activated Sepharose

Cyanogen bromide activated Sepharose 4B-CL (Pharmacia Fine Chemicals, Uppsala, Sweden), to be used for affinity chromatography after conjugation to protein antigen or antibody (Porath et al., 1967), was prepared as follows:

16 grams of a semidry filter cake of resin, washed with several volumes of distilled water by suction filtration on a Millipore funnel, were resuspended in 50 ml of a 2 per cent solution of CNBr (Eastman, Rochester, N.Y., USA), freshly prepared in distilled water. The reaction mixture was agitated by a magnetic stirrer and the pH adjusted to 10.5(± 0.5) by careful addition of a fresh solution of 1 M sodium hydroxide until 8 ml were consumed. The reaction was interrupted by adding crushed ice and the activated resin was immediately washed with ice cold 0.2 M sodium bicarbonate, pH 9.5 on a suction filter. After removal of excess buffer, the filter cake of activated Sepharose was resuspended in 10-40 ml of protein in 0.2 M sodium bicarbonate buffer, pH 8.5.

8. Chemicals

Standard chemicals were of reagent grade. Special chemicals and reagents needed for a particular assay or procedure are specified in the method section.

9. Enzymes

Glucose oxidase, Aspergillus niger, type II was purchased from Sigma Chemical Co., St. Louis, Mo., USA.

Histamine N-methyl transferase used in the histamine assay was prepared from guinea pig brain, essentially as described by Brown et al. (1959). Freshly frozen brains from guinea pigs were kindly supplied by Dr. P. Wåhlén, Institute of Clinical Bacteriology, University of Göteborg, Sweden. 50 grams of brain tissue suspended in 50 ml 0.25 M sucrose were homogenized in a Potter-Elvehjem homogenizer at 4°C. After centrifugation (20,000 x g, 20 min at 4-6°C), the supernatant was equilibrated against 1 mM phosphate buffer, pH 7.4, by dialysis at 4°C. The enzyme was precipitated by addition of 50 grams of solid ammonium sulphate per 100 ml supernatant at 4°C. After 60 min the precipitate was collected by centrifugation (20,000 x g, 10 min 4-6°C, dissolved in a small volume of distilled water and finally dialysed against 1 mM phosphate buffer, pH 7.4.

Lactoperoxidase (from Milk) was purchased from Sigma Chemical Co.

Pepsin (twice crystallized) from Swine stomach was obtained from Worthington Biochemical Corporation, Freehold N.J., USA.

Trypsin-Sepharose was prepared essentially as described by Cuatrecasas and Anfinsen (1971); 185 ml of Sepharose 4B-CL (Pharmacia Fine Chemicals, Uppsala, Sweden), carefully washed in distilled water, were suspended in 148 ml distilled water. 37 ml of 1 M sodium periodate were added and the reaction mixture was incubated with stirring for 2 hrs at RT. The mildly reduced resin was washed with 1 liter of 0.05 M ethylene glycol (E. Merck AG, Darmstadt, Germany) and 2 liters of distilled water, then resuspended in 185 ml 2 M ethylene diamine (E. Merck AG) pH 5. After 6 hrs at RT, the pH was adjusted to 9 with solid sodium carbonate followed by dropwise addition of 18.5 ml of a freshly prepared 5 M solution of $NaBH_4$. After 12 hrs at 4°C, the product (aminoethyl-Sepharose) was extensively washed with distilled water, and resuspended in 185 ml of 0.2 M sodium borate, pH 9.3 in 40 per cent dimethyl formamide (Mallincrodt). 14 millimoles of p-nitrobenzoylazide (Eastman, Rochester, N.Y., USA), dissolved in 20 ml dimethyl formamide, were added and the mixture was stirred for 4 hrs at RT. The product (p-nitrobenzamidoethyl-Sepharose) was extensively washed with 50 per cent aqueous dimethyl

formamide and 0.5 M sodium bicarbonate, pH 8.5, and finally reduced by reaction for 1 hr at 40°C with 185 ml of 0.2 M sodium dithionite in 0.5 M sodium bicarbonate pH 8.5. The product (p-aminobenzamidoethyl-Sepharose) was stored in 1 per cent sodium azide at 4°C before use. Prior to conjugation with protein, the p-aminobenzamidoethyl-Sepharose derivative was carefully washed with 0.5 M HCl then diazotized by reaction at 4°C for 7 min with an equal volume of 0.2 M sodium nitrite in 0.5 M HCl. Excess reagent was removed by aspiration and the diazonium derivative washed with a small volume of distilled water. The moist filter cake of resin was added to a 2.5 per cent (w/v) solution of trypsin (DCC-treated, Sigma Chemical Co), dissolved in saturated sodium borate, pH 8 and stirred gently for 24 hrs at 4°C. The resin was carefully washed with buffer and the amount of unbound trypsin calculated from the absorbance of the eluate at 280 nm, based on A280 nm, 1%, 1 cm of 14.3 for trypsin (Worthington Enzyme Manual, 1977). The preparation of trypsin-Sepharose contained about 12 mg enzyme per ml settled bed volume, corresponding to about 2.7 mg per g of semidry resin.

10. IgE producing rat immunocytomas

Two IgE secreting rat (LOU/C) immunocytoma lines - IR2 and IR162 - transplanted by intraperitoneal injections into syngeneic LOU/M rats, were kindly supplied by Dr. H. Bazin, University Louvain, Belgium. The tumour lines have been maintained by serial passage in LOU/C and/or LOU/M rats in the laboratory of Dr. H. Bennich, The Biomedical Center, Uppsala, Sweden, since 1975. Plasma and/or ascitic fluid collected from tumour carrying animals were used as starting material for isolation of monoclonal IgE.

11. Nippostrongylus brasiliensis parasites

Larvae and eggs from the helminth Nippostrongylus brasiliensis were kindly supplied by Dr. Ellen Jarrett, The Wellcome Laboratories for Experimental Pathology, University of Glasgow. The method used for maintaining the parasite in culture is described by Jennings et al. (1963).

12. Serum

Myeloma sera collected from LOU rats carrying immunocytomas (myelomas) secreting respectively IgM(IR202), IgE(IR2) and IgE(IR162) were kindly supplied by Dr. H. Bazin and used both as reference sera and as a source for the initial isolation of monoclonal IgE and IgM.

Normal rat serum. Pools of normal rat serum were obtained from apparently healthy nonimmunized adult rats of the Hooded Lister and the Sprague Dawley strains and used both as sources for the isolation of normal rat IgG and as diluents in various tests as described in the Method section.

Reference sera, containing specific IgE antibody, produced by immunization and/or parasitic infection of Hooded Lister rats, were kindly supplied by Dr. Ellen E. Jarrett, University of Glasgow, Scotland.

Reference serum no 1 (HL-anti-OA) was collected 4 days after a second injection i.p. of ovalbumin(OA); PCA-titer(OA) = 1024, and total IgE = 7 mg/l.

Reference serum no 2 (HL-anti-OA /N.b.) was collected 12 days after infection with Nippostrongylus brasiliensis larvae of OA-primed animals; PCA-titer(OA) = 1024, and total IgE = 120 mg/l.

Reference serum no 3 (HL-anti-N.b.) was collected 6 days after a second infection with Nippostrongylus brasiliensis larvae; PCA-titer = 2048, and total IgE = 125 mg/l.

Test serum, from nonimmunized adult rats of various strains kept under different animal housing conditions, was kindly supplied by Dr. H. Bazin (AxC,DA,Fischer,LOU/C,LOU/M,LOU/C/Ig4(OKA),Okamoto, Wistar); Dr. I. Heron, Aarhus University, Denmark (AS,Fischer,BN, HL and F1 hybrids Fi/AS,Fi/HL,Fi/BN,HL/AS,HL/BN,BN/AS); Dr. H. Wigzell, The Biomedical Center, Uppsala (Lewis).
Test serum from young (6 weeks old) rats of various strains, kept under conventional and SPF breeding conditions, respectively, were supplied by Møllegaard, Avlslaboratorium ApS, L. Skensved, Denmark (HL,Lewis,SHR,SD,Wistar).

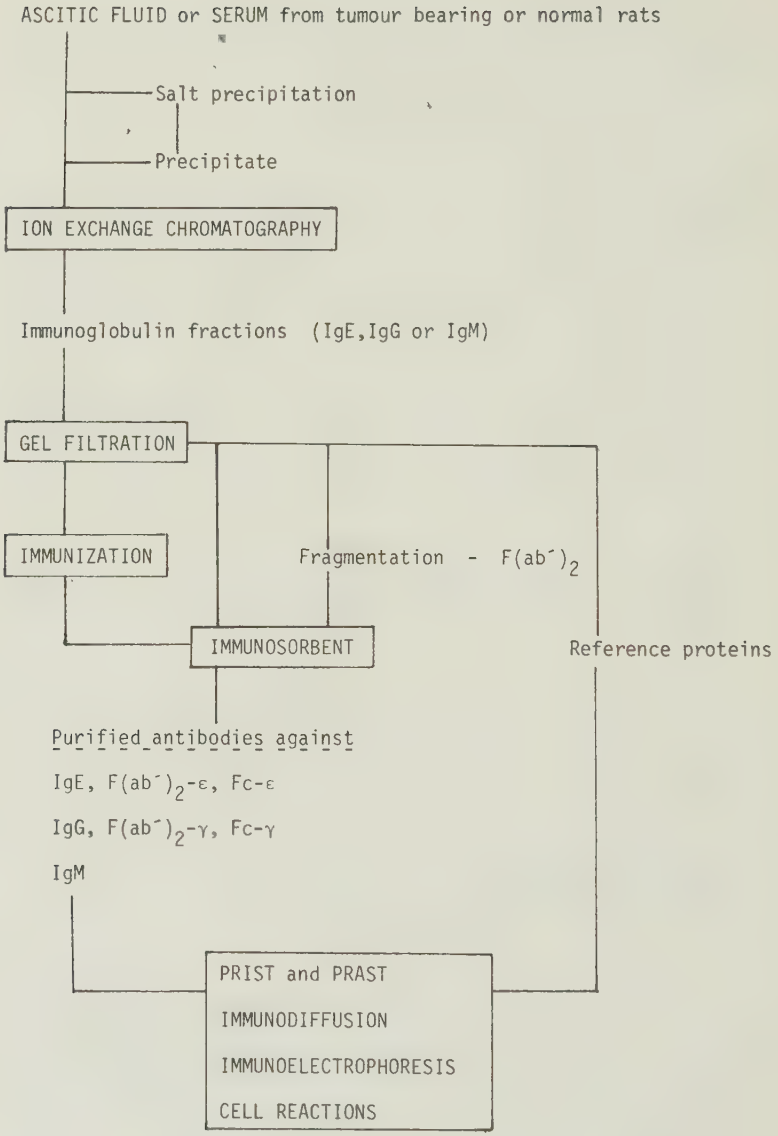


Fig. 2. Flow diagram of procedures for isolation of Ig(G,E,M) and purification of specific antibody for PRIST and PRAST.

III. ANALYTICAL AND PREPARATIVE METHODS

1. Isolation of rat immunoglobulin (Ig).

Monoclonal IgE and IgM and normal IgG were isolated from serum and ascitic fluid either by salt precipitation, ion exchange chromatography and gel filtration, in that order, or by ion exchange chromatography and gel filtration, as shown in Fig. 2. Ascitic fluid was defribrinated by dialysis against 0.1 M Tris-HCl pH 8 in the cold (4-6°C) followed by centrifugation at 40.000 x g for 15 min. Freshly collected serum was filtered through a glass filter (Whatman GF/A); frozen and thawed serum was centrifuged at 40,000 x g for 10 min prior to filtration in order to remove any fat and fine precipitates. To one volume of serum (or ascitic fluid), equilibrated at 20°C, one volume of 100 per cent saturated ammonium sulphate (SAS) (pH 6.5-7) was added dropwise with stirring. After incubation for about 60 min at 20°C, the precipitate was collected by centrifugation (20,000 x g, 15 min), washed with 50 per cent SAS, and recollected by centrifugation. The final precipitate was dissolved in a small volume of distilled water and equilibrated against 0.1 M Tris-HCl pH 8 by means of dialysis. Occasionally, salting-out of immunoglobulins by using sodium sulphate was preferred: To a sample of serum (or ascitic fluid) incubated in a water bath (25°C) was carefully added anhydrous sodium sulphate in small increments to give a final concentration of 18 g salt per 100 ml serum. After 60 min at 25°C, the precipitate was collected by centrifugation and washed with a solution of 18 per cent (w/v) sodium sulphate in distilled water. The final precipitate was dissolved in a small volume of distilled water and dialysed against 0.1 M TRIS-HCl buffer, pH 8 at 4°C.

Ion exchange chromatography was performed on a column (32 x 300 mm) of DEAE-Sephadex A-50 or DEAE-6BCL-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden), equilibrated in 0.1 M Tris-HCl buffer, pH 8.0 at 20°C. Samples (20-40 ml) of a dialysed solution of Ig isolated by salt precipitation (see above) or serum (or ascitic

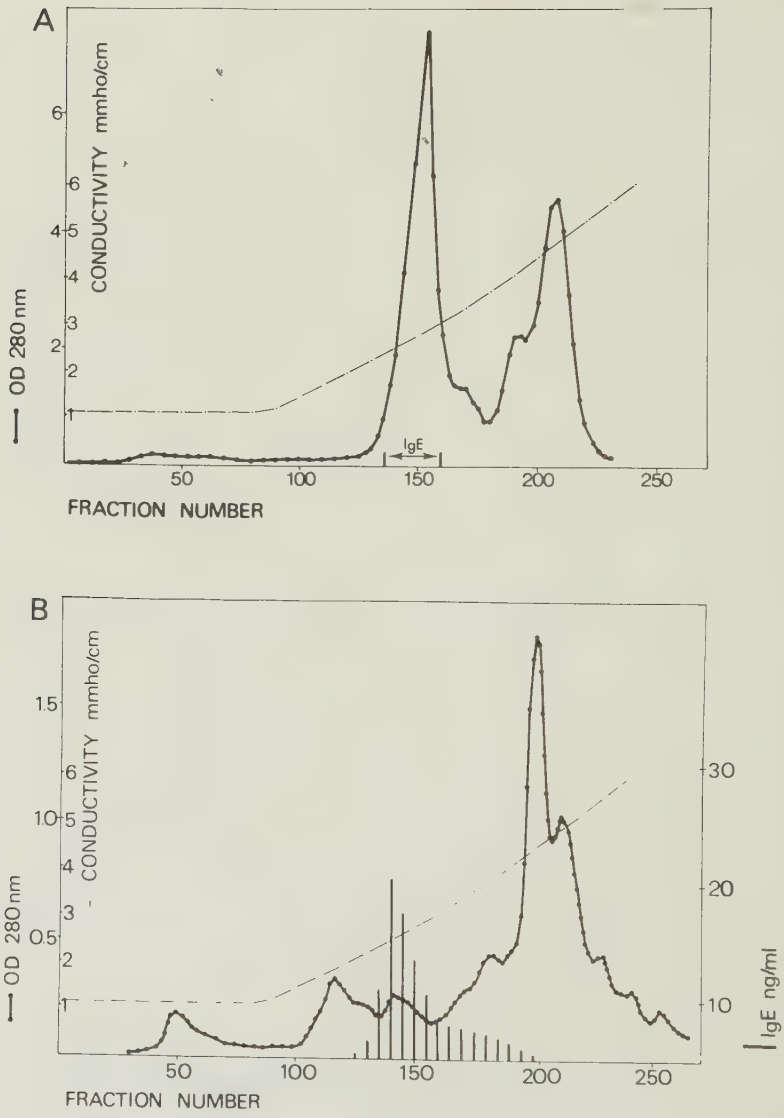


Fig. 3. Ion exchange chromatography of rat E myeloma serum IR2, (A); and serum from nonimmunized adult Hooded Lister rats, (B). Conditions: DEAE-6B-CL, Sepharose 32 x 300 mm; 0.1 M Tris-HCL pH 8.0(RT). Concave gradient (Ultrograd, LKB-Produkter AB, Sweden) from 0 to 0.7 M NaCl in Tris-buffer pH 8.0. Flow rate: 50 ml per hr. Fractions: 4 ml.

fluid) were applied at a flow rate of 30 ml per hr. Unadsorbed proteins were eluted by one column volume of starting buffer; adsorbed proteins were eluted by using a concave salt gradient, from 0 to 0.7 M sodium chloride in starting buffer, pH 8.0 created by means of an Ultrograd apparatus (LKB-Produkter AB, Bromma, Sweden). 4 ml fractions were collected, and the protein elution pattern was determined by spectrophotometry at 280 nm, using a MS2 Spectrophotometer (Micromedic Systems Inc., Pa., USA). The actual gradient was determined using a Tetramatic MHO-meter (Kemotron, Denmark). Fractions, found to contain immunoglobulin as determined by immunodiffusion, immunoelectrophoresis and SDS-polyacrylamide gel electrophoresis, were pooled and concentrated to a final protein concentration of 15-25 mg per ml. The elution pattern of samples of rat IgE myeloma serum and serum from a nonimmunized Hooded Lister rat are illustrated in Fig. 3. Final purification of Ig containing fractions was performed by gel filtration on a column (32 x 900 mm) of Sephadex G-200 or Ultrogel AcA-34 (LKB-Produkter AB, Bromma, Sweden) equilibrated in TBS-7.7 buffer. After application of 10-15 ml of sample, containing up to 150 mg of protein, the column was eluted with TBS-7.7 at a flow rate of 15 ml per hr (for Ultrogel AcA-34: 30 ml per hr). 4 ml fractions were collected and analysed as described above. The purity of the final product was assessed by immunodiffusion, immunoelectrophoresis and by SDS-polyacrylamide gel electrophoresis of both native and reduced samples.

2. Preparation of $F(ab)'_2$-ε.

$F(ab)'_2$-ε fragment was produced by tryptic cleavage of purified rat myeloma IgE. To 5 ml of a solution of IgE(IR2) (10 mg of protein per ml in TBS-7.7) was added 1 g of a semidry filter cake of trypsin-Sepharose conjugate (corresponding to approx. 3.7 mg of trypsin). The suspension was gently stirred for about 24 hours at 20°C. The digest was separated by suction filtration on a Millipore funnel (membrane, 1.2 μ). The resin was washed with 5 volumes of TBS-7.7, and the combined eluate and washings were concentrated to 5 ml. Analysis of the digest by SDS-PAGE indicated that only minute amounts (approx. 5%) of the original protein remained essentially intact. The $F(ab)'_2$-ε fragment was separated from peptides and remaining IgE by gel filtration on Ultrogel AcA-34, as described above (III.1).

3. Preparation of $F(ab)'_2$-γ.

$F(ab)'_2$-γ fragment was produced by peptic digestion of purified IgG, isolated from a serum from nonimmunized Sprague Dawley rats. To 5 ml of a solution of rat IgG (containing 10 mg/ml in 0.1 M sodium-acetate, pH 4.5) were added 50 µl of a 1 per cent (w/v) solution of pepsin dissolved in buffer. After incubation for 12 hours at 37°C a second aliquot (50 µl) of the enzyme solution was added and the digestion continued for another 12 hours. The reaction was interrupted by addition of solid Trizma-Base to a final pH of 8.5. Purification of the $F(ab')_2$-γ fragment was performed by gel filtration on Ultrogel AcA-34 as described above (III.1). SDS-PAGE analysis of the final product revealed a single component, yielding two band upon reduction, corresponding to the positions of light and Fd' polypeptides.

4. Preparation of immunosorbent.

8 grams of a semidry filter cake of CNBr-activated Sepharose 4B-CL (see II:7) were carefully suspended in 25 ml of a solution of antigen (or antibody) containing 2 to 5 mg of protein per ml in 0.2 M sodium bicarbonate, pH 9. After gentle stirring for 4-6 hrs at 4°C, the liquid phase was collected by suction filtration on a Millipore funnel to determine the amount of uncoupled protein. The semidry resin-protein conjugate was resuspended in 50 ml of 0.2 M sodium bicarbonate - 50 mM ethanolamine (redistilled), pH 9 in order to block any residual active sites. After 16 hrs at 4°C, the Sepharose-protein conjugate was carefully washed on a Millipore funnel with the following buffers to remove any adsorbed protein: 0.2 M sodium bicarbonate - 0.5 M sodium chloride pH 9 for 20 min; 5 M urea in 0.1 M soidum acetate pH 5 for 5 hrs; 0.1 M glycine-HCl, pH 3.0 containing 0.5 M sodium chloride for 1 hr. Finally, the immunosorbent was equilibrated against 0.1 M Tris-HCl, pH 8.0 containing 0.5 M sodium chloride.

5. Isolation and purification of specific antibody.

Antibody, specific to rat IgE, Fc-ε, $F(ab')_2$-ε, IgG and IgM respectively, was isolated by affinity chromatography on CNBr-activated Sepharose conjugated to the appropriate antigen.

Antibody specific to IgE (Rabbit-anti-rat-IgE): Rabbit antiserum (10-30 ml) to rat IgE(IR162) was allowed to react with normal rat IgG coupled to CNBr-activated Sepharose (32 x 20 mm, bed volume: 16 ml) to remove any contaminating antibody to light (kappa)chain determinants. The eluate, containing IgE class specific antibody and IgE(IR162) specific (idiotype) antibody was passed through a second column packed with 16 ml Sepharose-conjugated IgE(IR2) at a flow rate of about 5 ml per hr at 4-6°C. The column was washed with 10 volumes of TBS-7.7 and 2 volumes of 0.5 M sodium chloride before antibody was desorbed by elution with 0.1 M glycine-HCl-0.5 M NaCl, pH 3.0. The eluate was dialysed against 0.1 M Tris-HCl, pH 8, concentrated to a final concentration of 5-10 mg protein per ml, and finally purified by gel filtration on Ultrogel AcA-34 or Sephadex G-200 as described above (III:1). Fractions, containing monomeric antibody, were pooled and concentrated to a final protein concentration of 5-10 mg per ml and kept frozen at -30°C before use.

Antibody specific to $F(ab)'_2$-ε (Rabbit-anti-rat-$F(ab)'_2$-ε): Rabbit antiserum to rat IgE(IR162) was reacted with insolubilized rat IgG as described above. The combined eluate and washings were passed through a 16 ml column of Sepharose-conjugated rat $F(ab)'_2$-ε (IR2) at a flow rate of about 5 ml per hr at 4°C. Bound antibody, directed to antigenic determinant groups analogous to Dε1 on human IgE (see Fig. 1), was desorbed at pH 3 and purified as described above.

Antibody specific to Fc-ε (Rabbit-anti-rat-Fc-ε): The combined eluate and washings, obtained after the passage of rabbit antiserum to IgE(IR162) through a column of Sepharose-$F(ab)'_2$-ε (IR2) as described above, were applied to a second column containing Sepharose-IgE(IR2). Bound antibody, directed to antigenic determinants analogous to Dε2 on human IgE (see Fig. 1), were desorbed and purified by gel filtration as described above.

6. Isolation of mast cells.

Purification of mast cells, obtained by pleural and/or peritoneal washings from adult (3-5 mo.) Hooded Lister and Sprague Dawley rats, was done by using a one-step density gradient of BSA in PBS (Keller, 1966), rather than by using a Ficoll gradient (Bach et al.,

1971, Uvnäs and Thon, 1959) as the latter system was found to repeatedly yield purified cells of low viability and with impaired reactivity. The animals were anaesthetized with aether. Blood was collected from the tail vein prior to removal of abdominal skin and intraperitoneal injection of 10 ml of lukewarm PBS-2 mM EDTA. After gentle massage of the abdomen for 60 seconds, the peritoneal cavity was opened, and peritoneal cells collected with a polypropylene pipet. An additional volume of 10 ml of PBS-EDTA was injected and the two washings were combined. 5 ml of PBS-EDTA were then injected intrapleurally and pleural cells were collected as described above. Cell suspensions were kept on an ice bath until pelleted at 120 x g for 5 min in a Sorvall GLC centrifuge equipped with a HL-4 swing-out rotor. The cell pellets collected from 3 rats were resuspended in 1 ml of PBS, layered on top of 1 ml of a 29 per cent (w/w) solution of BSA in PBS in a polypropylene tube and finally centrifuged at 180 x g for 10 min at 4°C. Under these conditions, mast cells (and occasionally a few red cells) will sediment through the layer of BSA to the bottom of the tube, while other kinds of cells will remain at the interface. After removal of supernatant liquid, the mast cells were resuspended in 100-200 µl of PBS and washed twice in PBS. About 95 per cent of the mast cells present in the peritoneal and pleural washings were recovered. The total number of purified mast cells obtained from adult rats of the Hooded Lister and Sprague Dawley strains was 10^6 and 2×10^6, respectively. It should be mentioned that the recovery of purified mast cells from adult LOU rats was found to be only about 30 per cent using the above procedure.

7. Immunochemical analysis

Immunoelectrophoresis was performed according to the method of Grabar and Williams (1953) by using 1.5 per cent (w/v) agar (Special Agar-Noble, Difco Laboratories, Detroit, Michigan, USA) in barbital buffer pH 8.6, I = 0.05 and applying 14 V per cm. The experiment was interrupted when bromophenolblue-albumin, applied as marker in a separate well, had migrated about 3 cm towards the anode. The subsequent antibody-antigen reaction was allowed to proceed at RT,

and the plates were examined 12 and 24 hrs after addition of antiserum. Qualitative gel immunodiffusion analysis in two dimensions was performed in 1.5 per cent (w/v) agar (Special Agar-Noble) in 0.3 M phosphate buffer pH 8.0 (Rowe and Fahey, 1965) at RT. Quantitative gel immunodiffusion analysis was done by the single radial immunodiffusion method as described by Mancini et al. (1965).

8. Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gel electrophoresis (PAGE) was run in 0.3 x 7 x 13 cm gels (7.5 per cent acrylamide-0.6 per cent crosslinked) in 10 times diluted Fairbanks buffer pH 7.4 containing 0.2 per cent sodium dodecyl sulphate (SDS). The gels were prepared in a commercially available gel slab casting apparatus (GSC-8, Pharmacia Fine Chemicals, Uppsala, Sweden). Prior to electrophoretic analysis, 50 µl of either 18 per cent (w/v) SDS in 6 times diluted Fairbanks buffer or 18 per cent (w/v) SDS-6 per cent (v/v) 2-mercaptoethanol in 6 times diluted Fairbanks buffer, and incubated on a boiling water bath for 2 min. After cooling to RT, 10 µl of a solution of Pyronin Y (0.2 mg per ml in 50 per cent glycerol) were added. After pre-electrophoresis of unloaded gels at 2.5 V per cm for 1 hr using a Model GE-4 electrophoresis apparatus (Pharmacia Fine Chemicals) at 10°C, up to 12 samples, each containing 5-15 µg of protein in 3-10 µl were applied. After 5 hrs at 7.5 V per cm, or 12 hrs at 3 V per cm, the gels were removed for fixation in 50 per cent methanol - 10 per cent acetic acid for 15 min followed by staining in 0.02 per cent (w/v) Coomassie Brilliant Blue G (Sigma Chemical Company, St. Louis, USA) dissolved in fixing solution for 1-2 hrs and destaining over night in 10 per cent acetic acid. For autoradiography, destained gels were washed twice in 2 per cent (v/v) glycerol in distilled water for about 1 hr and finally dried on Whatman 1 filter paper at 45°C in a vacuum chamber. The position of reference proteins were marked with radioactive ink (^{35}S-Sodium Sulphate). The surface of the gel was protected by a layer of Vitafilm (Goodyear) and sandwiched to a 13x18 cm sheet of X-ray film (Kodirex, Kodak Ltd., London, England) for 12-48 hrs at RT.

9. Dialysis and ultrafiltration.
Samples of serum or protein solutions were dialysed in Visking

cellulose tubing, 23/32" (Union Carbide, St. Louis, USA) washed in 10 per cent acetic acid and distilled water or, in Spectrapor No 1 membrane tubing (Spectrum Medical Industries, Inc., Los Angeles, USA) that had been treated according to the manufacturers instruction. Dilute protein samples were concentrated by either vacuum filtration using Visking cellulose tubing 8/32", or pressure filtration in an Amicon pressure apparatus (Amicon Corporation, Massachusetts, USA) fitted with a UM-10 membrane.

10. Protein concentration measurements.
The concentration of isolated immunoglobulin or antibody was determined from the absorbances at 280 nm, in a Micromedic MS2 spectrophotometer (Micromedic Systems Inc., Pa., USA) using an extinction coefficient of A (1%, 1 cm) = 14.0.

11. Radioiodination procedures.
Iodination of protein in solution: Purified antibody or antigen in solution was radioiodinated by using the Chloramine T procedure (Hunter and Greenwood, 1962): To 50 µg of protein in 50 µl PBS were added 1 mCi ^{125}I (The Radiochemical Centre, Amersham, Bucks., England) and 50 µg Chloramine T dissolved in, respectively, 10 and 50 µl of 0.5 M phosphate buffer, pH 7.4. After 60 seconds, 100 µg of $NaHSO_3$ in 50 µl phosphate buffer, pH 7.4 were added, followed by 50 µl of saturated KI in water and 10 µl of saturated KI in water and 10 µl of an one per cent (w/v) solution of bromophenol blue in 0.05 M phosphate buffer pH 7.4 containing 0.3 per cent (w/v) of bovine serum albumin (BSA). Albumin bound bromophenol blue served as a marker during the subsequent purification of the reaction mixture by gel filtration on Sephadex G-200 (0.9 x 60 cm, 0.3 per cent BSA in 0.05 M phosphate buffer pH 7.4). Fractions containing radiolabelled monomer of antibody (or antigen) were pooled and distributed in small aliquots kept at -30°C until further use. For PRIST and PRAST only those preparations of radiolabelled protein having a labelling efficiency of 80-90 per cent or, in the case of radiolabelled antibody, having an immune reactivity of at least 50-80 per cent, as measured by solid phase absorption experiments, were used. One nanogram of antibody represented 10,000 - 20,000 counts per min.

Iodination of membrane associated proteins: Radiolabelling of integral membrane protein, or protein bound to receptor molecules on the surface of intact cells, was performed by the lactoperoxidase method (Hubbard and Cohn, 1972). A suspension of 10^7 washed cells in 200 µl PBS was carefully mixed with 100-200 µCi ^{125}I (or ^{131}I) in 20 µl PBS, 450 µg of glucose in 50 µl PBS and 30 µg of glucose oxidase in 50 µl PBS. After 30 seconds 10 µg of lactoperoxidase in 50 µl PBS were added. After 5 min incubation at RT a second portion of glucose, glucose oxidase and lactoperoxidase was added, as described above, and the reaction mixture incubated for another 5 min at RT. Finally, the cells were carefully washed 3 times in PBS.

12. Immunization of rats.

Groups of 3-5 rats were immunized by using one of the following protocols:

a) Antigen (10-500 µg) injected (i.p.) with simultanous injection (s.c.) of 2,000-4,000 larvae of Nippostrongylus brasiliensis using the techniques for preparation of larval dose and infection as described by Jarrett et al. (1968).

b) Antigen (10-500 µg) emulsified in 100 µl Freund´s complete adjuvant injected (s.c.).

c) Antigen (10 µg) adsorbed to 10 mg aluminium hydroxide gel injected (i.p.).

The protocol to be used for a particular series of immunization was determined by the purpose of the experiment and no complete study of the optimal conditions for immunization was attempted.

13. Quantitative radioimmunoassay of total serum IgE.

Quantitative analysis of serum IgE in the rat was performed by an assay which is similar to the Paper Radio Immuno Sorbent Test (PRIST) originally developed for determination of human IgE (Ceska and Lundkvist, 1972). As illustrated in Fig. 4, PRIST is based on a sandwich procedure in which sample IgE (antigen), after binding to solid phase linked antibody, is identified by reaction with a second, radiolabelled antibody. Since the class specific antigenic structure for rat IgE has not yet been defined in terms of

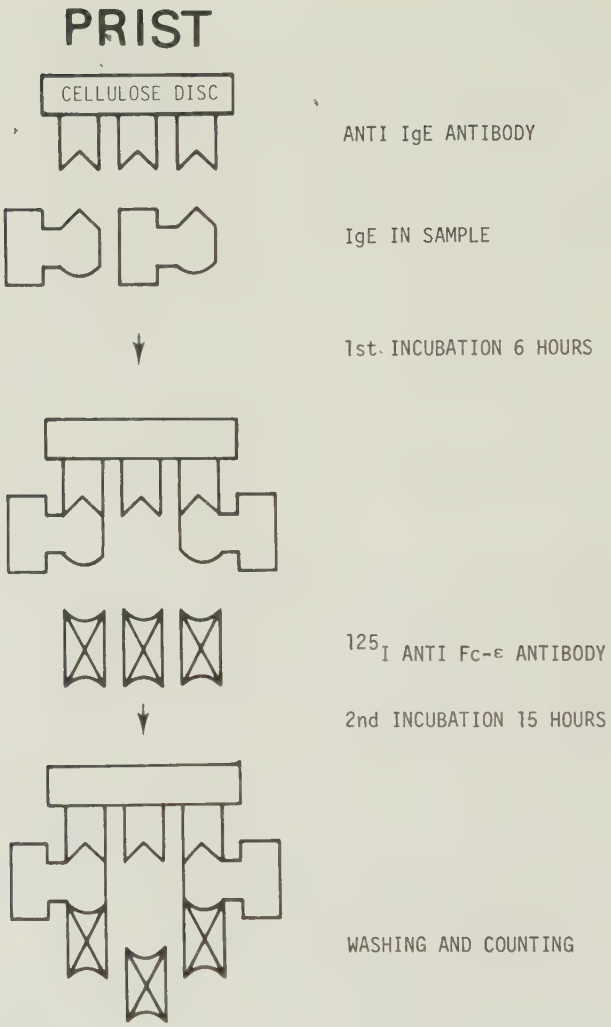


Fig. 4. Diagrammatic presentation of the paper-radio-immuno-sorbent-test (PRIST) for total IgE.

activity and distribution of antigenic determinant groups on isolated, well-characterized enzymic fragments, as has been done for human IgE (see Fig. 1), two kinds of operationally defined antibodies were compared for determination of rat IgE:

a) Anti-IgE, i.e. antibody reacting with antigenic determinants present within the constant regions of native rat epsilon chain; and b) Anti-Fc-ε, i.e. antibody reacting with native IgE but not with $F(ab')_2$-ε fragment, which indicates that the reaction involves antigenic determinant groups located within the carboxy-terminal (Fc) portion of rat epsilon chain.

A. Preparation of antibody coated paper discs.

Two grams of dry cyanogen bromide activated (see II:6) paper discs (approx. 1.000) were suspended in 40 ml 0.1 M $NaHCO_3$-10 mM Na_2CO_3, pH 9, containing 3-4 µg per ml of immunosorbent purified antibody (rabbit-anti-rat IgE, see III:5), and incubated with gentle stirring for 4 hrs at 4°C. The liquid phase was recovered by suction, the antibody coated discs were washed twice with fresh buffer, pH 9, and resuspended in 40 ml of buffer pH 9, containing 50 mM ethanolamine (redistilled). After incubation for 4-6 hrs at 4°C to block any residual active sites, the discs were washed twice for 10 min at RT in 100 ml of 0.1 M sodium acetate pH 4, resuspended in 'assay' buffer (see II:5) and stored at 4°C until use.

B. Assay procedure.

Prior to use the antibody coated discs were washed twice in 'assay' buffer to remove any breakdown products formed during storage. Before transfer to a series of disposable polystyrene tubes (11x80 mm), 1 paper disc per tube, excess buffer was removed by spreading the discs on a sheet of clean filter paper. 150 µl of sample, or dilutions of a solution of IgE standard, were added per tube; unknown samples and IgE standard dilutions were assayed in duplicate, together with a series of controls consisting of

10 per cent normal rat serum (Sprague Dawley) to allow correction for nonspecific uptake of radiolabelled antibody. After incubation for 6 hrs at RT, the liquid phase was removed by suction and the tubes washed twice for 10 min with 3 ml 'assay' buffer. 150 µl of ^{125}I-labelled antibody (purified rabbit anti-rat Fc-ε, see III:6), about 10 ng protein corresponding to 50,000-100,000 cpm in 'assay' buffer, were added to each tube. After incubation for 15 hrs at RT, the liquid phase was removed and the tubes washed twice in 0.15 M sodium chloride. The amount of radioactivity that remained bound was determined in a gamma-scintillation spectrometer (Modumatic VI Auto-Gamma, Model 5260) connected on-line to a programmable computer (Alpha 327 Scientist; Compucorp, Los Angeles, Calif., USA). Results were expressed as ng per ml as calculated from the simultaneously measured radioactive uptake for a series of standard dilutions of IgE, corrected for nonspecific uptake of radiolabelled antibody in the controls.

C. Preparation of IgE standard.

A stock solution of purified monoclonal rat IgE(IR2) was used throughout the present investigation for preparation of standard for PRIST. To mimic the experimental conditions for the assay of unknown samples, normal serum from young (4-6 weeks) Sprague Dawley rats was used as diluent. A series of standard dilutions containing 2, 4, 10, 20, 40, 100, 200 and 400 ng per ml of IgE(IR2) was prepared in 10 per cent normal Sprague Dawley serum. A standard curve for IgE as determined by PRIST is shown in Fig. 5.

14. Radioimmunoassay of serum IgE antibody.

Determinations of serum IgE antibody in the rat were performed by a radioimmunoassay, in the following referred to as PRAST (Paper Radio Allergo Sorbent Test), which is in principal similar to the procedure for measuring human IgE antibody to allergen immobilized to a solid support, as described by Wide et al. (1967 and 1972). A schematic illustration of the procedure used in the present study is shown in Fig. 6.

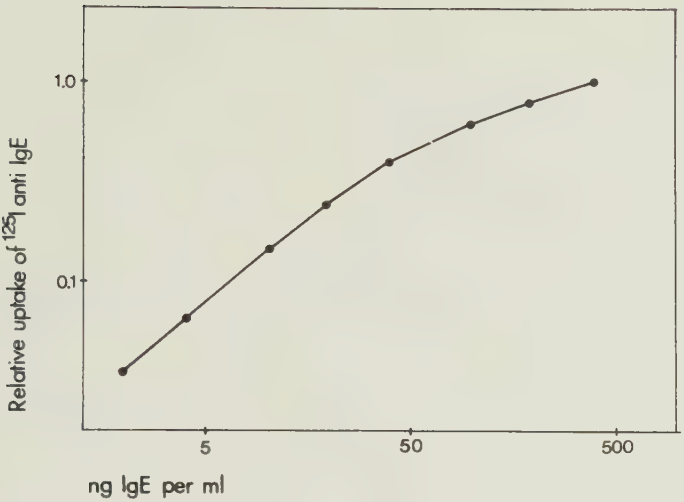


Fig. 5. Example of a standard curve for PRIST.

A. Preparation of antigen coated paper discs.

The procedure used for conjugation of antigen to CNBr-activated paper discs was identical to the procedure described above for preparation of antibody coated discs (III:13A) except that the amount of protein (antigen) was varied between 0.5 and 5 mg per 100 discs in 10 ml buffer, depending on the purity of antigen and/or the purpose of a particular experiment.

B. Assay procedure.

Antigen coated paper discs were washed twice in 'assay' buffer, dried on filter paper and transferred to disposable polystyrene test tubes (11x80 mm). To each tube 150 µl of sample, diluted 3 to 15 times in 'assay' buffer, was added. All samples, including standard serum and controls, were assayed in duplicate. As overly high values might be obtained for serum samples containing large amounts of total IgE, e.g. serum from parasite infected animals, appropriate dilutions of sample were also added to control discs, coated with bovine serum albumin,

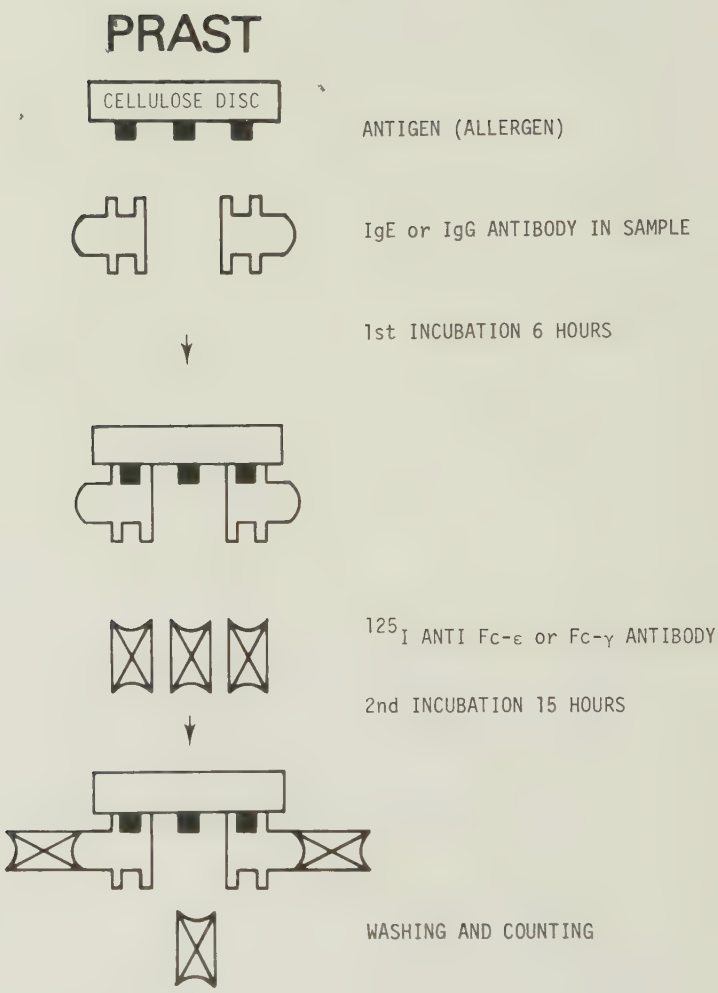


Fig. 6. Diagrammatic presentation of the paper-radio-allergo-sorbent-test (PRAST) for IgE and IgG antibody.

for correction of nonspecific uptake of radioactivity. After 6 hrs incubation at RT, the liquid phase was removed and the discs washed twice for 10 min in 3 ml 'assay' buffer. 150 µl of ^{125}I-labelled purified rabbit anti-rat Fc-ε in 'assay' buffer (10 ng of antibody protein giving 50,000 - 100,000 cpm) were added to each tube. After 15 hrs incubation at RT the liquid phase was removed and the tubes washed twice for 10 min in 3 ml 0.15 M NaCl. The uptake of radiolabelled antibody was measured in a gamma scintillation spectrometer (Packard Model 5260, 3" NI detector).

C. Standards for IgE antibody.

Antigen positive sera, having a known PCA-titer and total IgE level, were used as reference standards. Standard dilutions were made in normal serum, collected from healthy non-immunized Hooded Lister rats, in order to keep constant all serum factors other than IgE antibody.

15. Determination of serum IgE antibody by passive cutaneous anaphylaxis (PCA).

Passive cutaneous anaphylaxis (PCA) was introduced by Ovary (1952) as a sensitive procedure for evaluation in vivo of reaginic activity in the serum of antigen sensitized animals. The method is based on the observation that a normal animal, given an intradermal injection of reaginic serum, will respond by a reaction of local anaphylaxis given an intravenous injection of antigen 1 to 2 days after primary injection of reaginic serum. In terms of modern concepts, the PCA reaction consists of a series of events: First, a specific binding of IgE antibody (reagin) via its Fc region (Stanworth et al., 1968) to receptor molecules on the mast cell membrane (Ishizaka et al., 1970a); second, the bridging of membrane bound IgE antibody by multivalent antigen (Ishizaka et al., 1970b), which by an as yet unknown mechanism, provides the starting signal for a series of intracellular reactions resulting in the release of histamine and other vasoactive amines.

Procedure

Normal adult rats, shaved on the dorsal side, were lightly anaesthetized with aether. 50 µl of serial dilutions of serum in PBS (1/1, 1/2, 1/4 etc.) were injected intracutaneously at

duplicate sites. After 24 to 48 hrs, 0.5-1 ml of a 1 per cent solution of Evans' Blue in PBS, containing 2-3 mg of the antigen, was injected i.v. in the tail. After 20 min primary injection sites were examined and the extent of the blueing reactions was evaluated. A blueing reaction having a diameter of at least 5 mm was considered positive, and the highest serum dilution giving a positive reaction was recorded as the PCA-titer of that serum. Finally, the animals were killed and the skin examined from the inside for confirmation of the results.

16. Radioimmunoassay of IgG antibody.
Determination of serum IgG antibody in the rat was performed by PRAST using immunosorbent purified ^{125}I-labelled rabbit anti-rat IgG as the second antibody. The procedure for assay and for preparation of antigen coated paper discs were the same as those used for IgE-PRAST as described above (III:14). Since IgG-PRAST was developed for the determination of IgG antibody relative to the IgE antibody response in immunized rats and rats infected with parasite, no attempts were made to standardize the assay.

17. Cell incubation conditions for histamine release.
The conditions for induction of histamine release from rat mast cells were as follows:
50 µl samples of 5-6x10^4 purified mast cells (Hooded Lister and Sprague Dawley) suspended in MCM-7 buffer, were added to 300 µl of MCM-7 buffer in polypropylene tubes containing histamine releasing agents. After incubation at 37^{o}C for 15 min, 1.65 ml MCM-7 buffer was added and the tubes centrifuged at 150 x g for 1 min. 1.0 ml of the supernatant was collected and aliquots of 50 µl taken for analysis of histamine in solution. The 1 ml of supernatant remaining in the tube, including the cell pellet, was frozen and thawed for release of remaining cell bound histamine and after centrifugation at 10,000 x g for 2 min, aliquots of 50 µl were taken for histamine assay. The amount of histamine released (HR), expressed in per cent of the total amount of histamine present in the sample, was calculated as HR = (2A/A+B)x100, where A represents 1 ml

of the supernatant fraction without cells, and B the remaining 1 ml supernatant fraction including the cell pellet.
The experiments were performed in duplicate or triplicate. Cells incubated in MCM-7 buffer without histamine releasing agents were used as controls.

18. Histamine assay.

The classic procedure for analysis of histamine in solution utilizes the physiological effect of histamine on smooth muscle by measurements of the contraction of isolated guinea pig ileum (Guggenheim and Löffler, 1916, Barsoum and Gaddum, 1935). As the procedure is difficult to standardize and also is very tedious, chemical methods have been developed, which by utilizing the fluorescence (Shore et al., 1959) or the radioactivity (Snyder et al., 1966) of a histamine derivative, are more suitable for analysis of small differences in histamine content in a great number of samples. In the present study, histamine, released from rat mast cells, was measured by a single radioisotope method by modification of the procedure of Kobayashi and Maudsley (1972). The method is based on enzymic transfer of the methyl-^{3}H group in S-adenosyl-methionine (SAM-^{3}H) to histamine by means of histamine-N-methyl transferase. By the difference in solubility of SAM-^{3}H and methyl(^{3}H)-histamine in a two-phase system of an organic solvent and water, the radiolabelled histamine can be separated for analysis.

Assay procedure.

50 µl of sample in MCM-7 buffer was transferred to screw-capped centrifuge tubes containing 50 µl of SAM-^{3}H(250 µCi/mMole-400 pMole/ 50 µl, prepared by dilution of SAM-^{3}H (10 Ci/mMole, NEN Chemicals GmbH, W-Germany) with unlabelled SAM-chloride (Grade II, Sigma Chemical Company) and 50 µl of an appropriate dilutions of N-methyl transferase in buffer. After incubation at 37^{o}C for 2 hrs, the reaction was stopped by addition of 200 µl of 2 N sodium hydroxide saturated with sodium chloride to increase the density of the water phase. 4.5 ml of ethylacetate were added and the two phases carefully mixed by shaking for 30 sec. After centrifugation at 1.000 x g for 2 min at RT, 4 ml of the top(ethylacetate) phase, containing

methyl(^{3}H)-histamine, were transferred to a 20 ml scintillation vial (polypropylene) and evaporated to dryness by vacuum (water pump) at 45°C. 10 ml of scintillation liquid (5 g PPO,0.1 g POPOP in 1 L of toluene) were added and the radioactivity measured in a beta scintillation spectrometer (Model LS-250, Beckman Instruments Inc., Calif., USA). Samples and standards (histamine dihydrochloride, Sigma Chemical Company, USA) were run in duplicate or triplicate. A standard curve illustrating the range and sensitivity of the histamine assay is shown in Fig. 7.

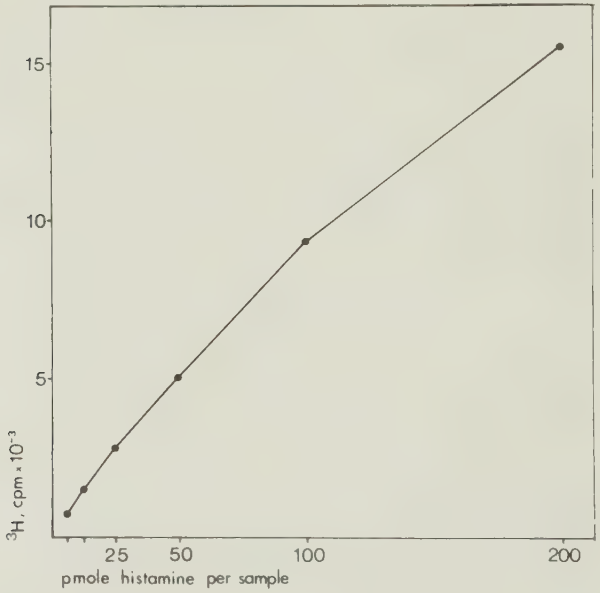


Fig. 7. Standard curve for histamine assay by a single radioisotope method. Histamine in the sample is converted to (^{3}H)-methyl-histamine using N-methyl transferase and radiolabelled (methyl-^{3}H) S-adenosyl-L-methionine and extracted with ethyl acetate for measurement of radioactivity.

19. Analysis of mast cell membrane components.

A. Solubilization

A pellet containing 1-5 x 10^6 purified rat mast cells (Hooded Lister or Sprague Dawley) that had been radiolabelled with ^{125}I-IgE(IR2) was resuspended in 1 ml NP40-PBS and incubated at 20°C for 15 min. After centrifugation at 20,000 x g for 10 min, both supernatant and pellet fractions were collected for analysis by SDS-PAGE (III:8) and/or SDS-gel filtration (see below) or, with respect to the supernatant fraction only, by gel filtration on Sepharose 6B in NP40-buffer (see below). In some experiments, radiolabelled cells were directly solubilized in SDS: A cell pellet containing 1-5 x 10^6 cells resuspended in 0.5 ml SDS-Fairbanks buffer, pH 7.4, was mixed with 0.1 ml 18 per cent SDS and incubated at 100°C for 10 min. After centrifugation (20,000 x g, 10 min), 50 µl of supernatant were taken for analysis by SDS-PAGE after mixing with 10 µl of Pyronin Y (0.2 mg per ml in 50 per cent glycerol), while 0.5 ml of the remaining supernatant fraction was diluted to 1 ml with SDS-Fairbanks buffer and subjected to analysis by SDS-gel filtration as described below.

B. Separation

1. SDS-PAGE was performed as described in III:8. For pretreatment of intact cells or the pellet obtained after centrifugation of NP40 extracts with SDS, the time for incubation at 100°C was increased to 10 min to insure solubilization of otherwise interferring cytoplasmic or nuclear components.

2. SDS-Gel filtration was performed on a 14 x 900 mm column of Ultrogel AcA-44 (LKB-Produkter AB, Bromma, Sweden) in SDS-Fairbanks buffer pH 7.4 at RT. Samples (1 ml) of solubilized membrane components, pretreated with 3 per cent SDS at 100°C for 10 min, were chromatographed at a flow rate of 6 ml per hr. Fractions of 1 ml were collected and analysed in a gamma scintillation spectrometer (Packard Model 5260).

3. Sepharose 6B in 0.5 per cent NP40-Tris buffer pH 8 was used for

the separation of mast cell membrane-IgE complexes. 1 ml of NP40-supernatant fraction was applied on a 14 x 950 mm column and chromatographed at flow rate of 6 ml per hr at RT. 1 ml fractions were collected for analysis in a gamma scintillation spectrometer (Packard Model 5260).

C. Immunoprecipitation of mast cell membrane-IgE complexes:

^{125}I-labelled Hooded Lister mast cells were solubilized in NP40-PBS as described above (III:19A).

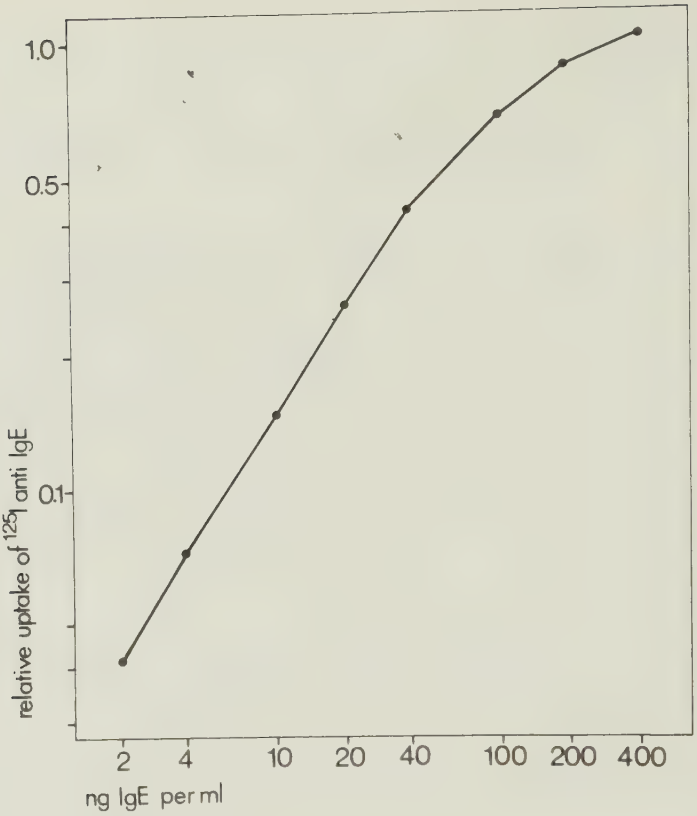
To 0.5 ml of NP40 supernatant containing 100 µg IgE(IR2), 100 µg of immunosorbent purified rabbit anti rat IgE(IR162) in 0.2 ml PBS was added. After incubation for 30 min at 37^{o}C, an equivalent amount of goat anti rabbit IgG in 0.3 ml PBS was added and the incubation was continued for another period of 30 min at 37^{o}C. The resulting immunoprecipitate was isolated by centrifugation (20,000 x g, 2 min), dissolved in 0.6 ml of 3 per cent SDS and finally diluted to 1.0 ml with SDS-Fairbanks buffer and subjected to analysis by SDS-gel filtration as described above (III:19B).

IV. RESULTS

1. Evaluation of the optimal conditions for quantitative measurements of rat IgE by PRIST

Construction of a standard curve for IgE-PRIST: The relation between uptake of radiolabelled anti-rat IgE and the concentration of IgE in a series of standard dilutions can be expressed in a number of ways. One method, illustrated in Fig. 8, is to express the values for each standard dilution relative to the radioactive uptake obtained at the highest concentration of IgE (in the present example, 400 ng per ml). Although several preparations of purified IgE(IR2), antibody coated discs and radiolabelled rabbit anti-rat IgE(IR162) have been used, the standard curve was found to remain constant over a long period of time.

Influences of times for incubation and the amount of radiolabelled antibody: By using standard experimental conditions with respect to antibody and reference protein, the variables that may influence the final results are the times for incubation and the amount of radiolabelled anti-IgE added to each sample. Fig. 9 illustrates the effect on the uptake of sample IgE by variation of the 1st incubation period between 3 and 12 hrs, keeping the 2nd incubation period constant at 20 hrs. Although the uptake increases with time, and, as would be expected, is particularly evident at low concentration of antigen, the differences are small. 6 hrs were regarded as being an appropriate time to chose for the 1st incubation in routine measurements of IgE. The effect of time, from 5 to 20 hrs, on the incubation with radiolabelled antibody is shown in Fig. 10. Again the differences were found to be small, but for routine measurements a 2nd incubation period of about 15 hrs was selected as this would allow the samples to be incubated over night. To determine what effect the amount of antibody added per sample had on the standard curve, a series of standard dilutions of IgE were tested by using identical experimental conditions except for the amount of radiolabelled anti-rat IgE. Fig. 11 illustrates that the



	Uptake of ^{125}I anti IgE			
ng IgE per ml	cpm	S.E.M.	% of added	relative
2	980	118	1.23	0.04
4	1681	159	2.10	0.07
10	3410	208	4.26	0.14
20	6034	343	7.54	0.26
40	9903	287	12.37	0.42
100	15643	412	19.55	0.66
200	20256	606	23.32	0.86
400	23680	1036	29.60	1.00

Fig. 8. Standard curve for PRIST. Mean values for 10 assays of rat IgE(IR2) 1st incubation: 6 hrs at RT; 2nd incubation: 15 hrs at RT. To each sample 11 ng (80,000 cpm) of ^{125}I-anti-rat IgE(IR162) were added. Background values (buffer only), corresponding to 1.1 per cent of added radioactivity, have been subtracted.

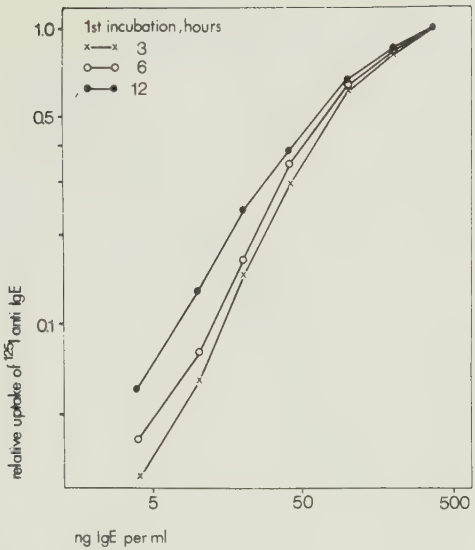


Fig. 9. Standard curve for determination of rat IgE by PRIST. Effect of time of the 1st incubation period (3, 6 and 12 hrs at RT). 2nd incubation: 20 hrs at RT.

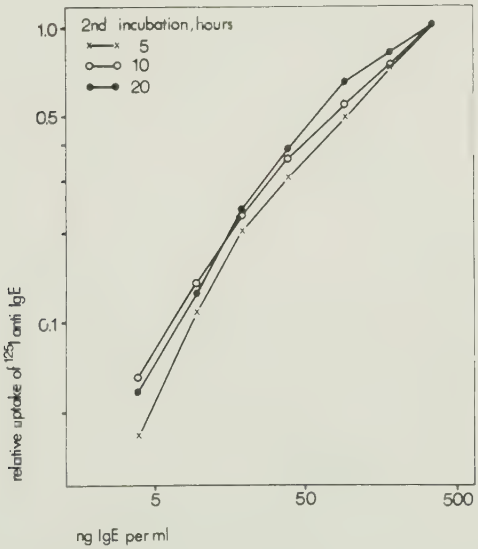


Fig. 10. Standard curve for determination of rat IgE by PRIST. Effect of time of the 2nd incubation period (5, 10 and 20 hrs at RT). 1st incubation: 12 hrs at RT.

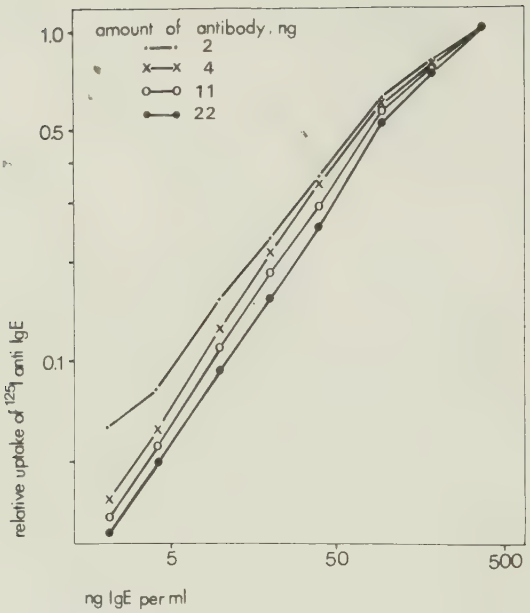


Fig. 11. Standard curve for determination of rat IgE by PRIST. Effect of the amount of ^{125}I-labelled antibody added per sample (2, 4, 11 and 22 ng rabbit anti-rat-IgE). 1st incubation 6 hrs and 2nd incubation 15 hrs at RT.

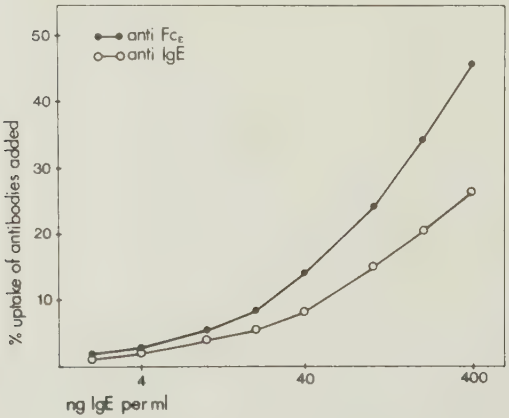
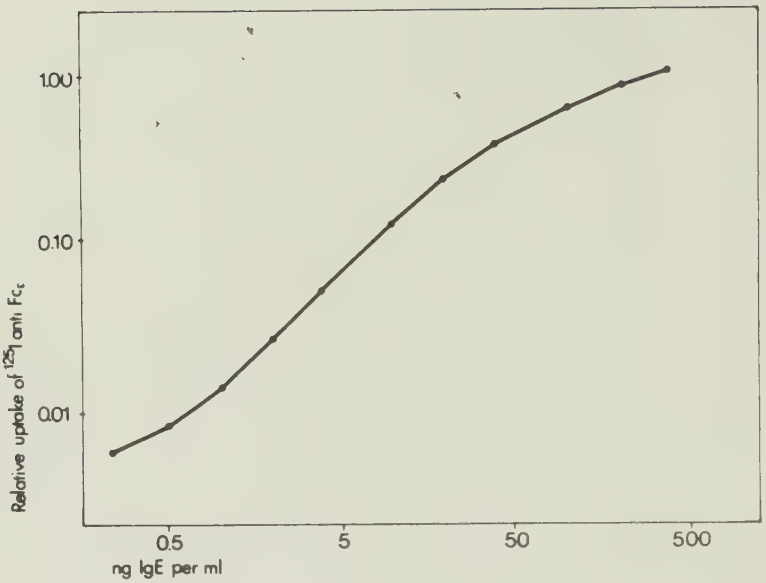


Fig. 12. The effect of the specificity of the second antibody on the sensitivity of PRIST. Comparison between ^{125}I-labelled rabbit anti-rat IgE and rabbit anti-rat Fc-ε.

standard curves obtained by 4, 11 or 22 ng of antibody per sample are parallel and very close to each other over the entire concentration range. Taking into consideration the specific activity and the immune-reactivity of radiolabelled antibody (in the present case 1 ng of antibody, corresponding to about 10,000 cpm having an immune-reactivity of 65 per cent), addition of 5-10 ng antibody per sample, corresponding to 50,000-100,000 cpm, appeared optimal for measurements of rat IgE in the range of 2-400 ng per ml.

In the experiments described above, immunosorbent purified anti-rat IgE was used both as the antibody coupled to paper discs and as the second, radiolabelled, antibody. The structure of rat IgE is not known in detail as yet, however, by assuming that human and rat IgE have a similar antigenic structure, the use of anti-rat IgE would be analogous to using a mixture of antibody to Dε1 and Dε2 for determination of human IgE by PRIST. In human IgE, Dε1 and Dε2 represent antigenic determinant groups located on the Fc-fragment, which is produced by digestion of IgE with papain (see Fig. 1). Since all attempts to produce an Fc-fragment from rat IgE by digestion with a variety of proteolytic enzymes have failed so far, rabbit anti-rat Fc-ε antibody directed to antigens within the carboxyterminal half of the rat epsilon chain has been prepared by solid phase absorption as described in III:5. A comparison of anti-IgE and anti-Fc-ε when used as the 2nd, radiolabelled, antibody in PRIST revealed, as illustrated in Fig. 12, that the radioactive uptake increased by 60-70 per cent by using anti-Fc-ε while the non-specific uptake remained at the same level as for anti-IgE (1.0-1.2 per cent of antibody added). Thus, by using anti-Fc-ε the sensitivity of PRIST is greatly enhanced and, as illustrated in Fig. 13, should allow measurements of rat IgE levels down to 0.25 ng per ml.

Evaluation of a one-step procedure of IgE-PRIST: In order to shorten the assay time and to avoid tedious washings between the 1st and 2nd incubations, the effect on the sensitivity of IgE-PRIST of using a one-step incubation procedure was studied.



ng IgE/ml	Uptake of ^{125}I anti Fc-ε		
	cpm	% of added	Relative
0.25	296	0.24	0.006
0.5	415	0.33	0.008
1	601	0.48	0.012
2	1367	1.09	0.027
4	2827	2.26	0.055
10	6356	5.07	0.124
20	11941	9.53	0.232
40	23108	18.44	0.450
100	30180	24.08	0.587
200	41806	33.36	0.814
400	51381	41.01	1.000

Fig. 13. Standard curve for determination of serum IgE by PRIST using ^{125}I-labelled rabbit anti-rat Fc-ε. 1st incubation: 6 hrs, 2nd incubation: 15 hrs at RT. Fifteen ng (130.000 cpm) of antibody were added per sample. Background (buffer) values, corresponding to 1.2 per cent of added radioactivity, have been subtracted.

Samples and radiolabelled antibody were added together to tubes containing antibody coated discs and the mixture incubated for 6 hrs at RT. The results were compared with those obtained with samples and radiolabelled antibody run in parallel but incubated separately for 6 and 15 hrs respectively. As shown in fig. 14, the two-step procedure for PRIST gave a higher uptake of radioactivity for all concentrations of the standard. Though a much higher precision would be obtained by increasing the incubation time, a one-step procedure, using the conditions as described above, would give sufficiently accurate values for IgE to make the method very useful for rapid screening of a great number of samples.

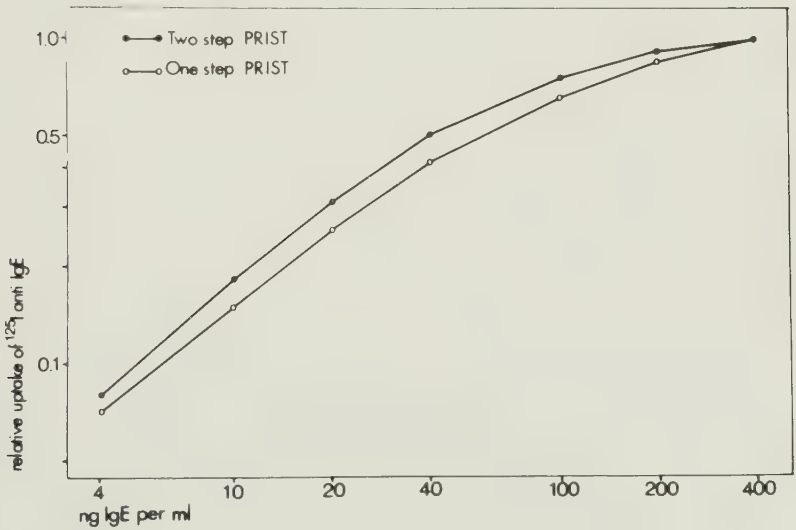


Fig. 14. Comparison of the sensitivity of PRIST, performed as a one-step and a two-step procedure. In the one-step procedure, sample and radiolabelled (^{125}I) antibody (anti-rat Fc-ε) were mixed and incubated for 6 hrs at RT; in the two-step procedure, sample and ^{125}I-antibody were added in sequence using 6 hrs for the 1st and 15 hrs for the 2nd incubation.

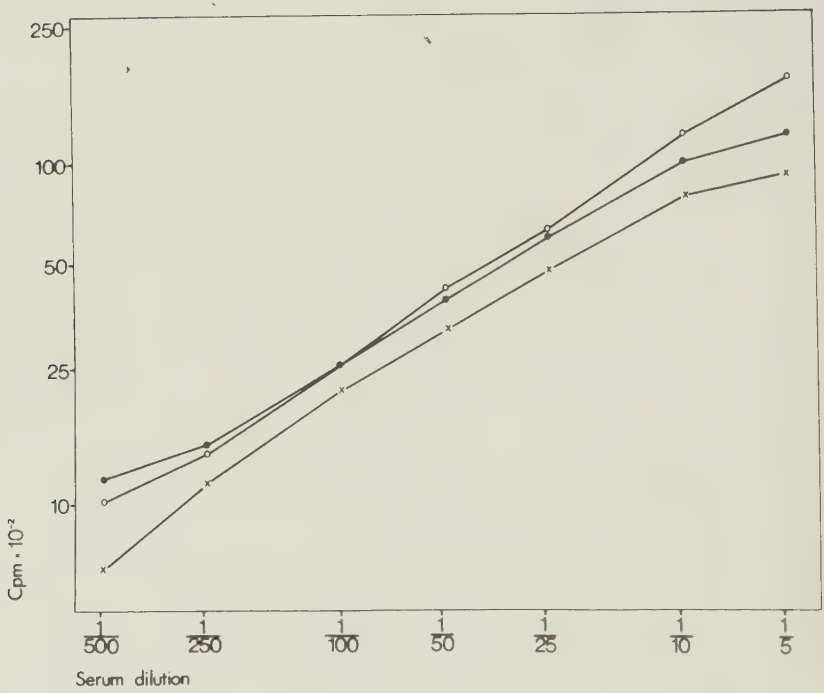


Fig. 15. Standard curve for rat IgE antibody determined by PRAST, using 3 different standard sera.
Reference serum 1(HL-anti-OA), PCA-titer(OA = 1024, total IgE = 7 µg per ml (●----●); Reference serum 2(HL-anti-OA /N.b./), PCA titer(OA) = 1024, total IgE = 120 µg per ml (o----o); Reference serum 3(HL-anti-N.b.), PCA-titer = 2048, total IgE = 125 µg per ml (x----x).

2. An approach to quantitative measurements of rat IgE antibody by PRAST.

By using passive cutaneous anaphylaxis (PCA), the classical in vivo method for evaluating the presence of antigen specific IgE (reaginic antibody) in an immune serum (Ovary, 1958), reactions by tissue cells other than mast cells might influence the outcome of both the primary reaction between IgE antibody and mast cell receptors and secondary reactions elicited by the sensitized mast cell. The principle of PRAST, on the other hand, is based on a straight forward in vitro reaction between antigen and antibody and does not, a priori, involve any uncontrollable parameters. By using PRAST for determination of the IgE antibody response in immunized or parasite infected animals it was found, as will be demonstrated in this thesis, that the results compare well with those obtained by PCA.

However, as indicated by preliminary studies, the utility of PRAST would be much improved if the procedure could be standardized and the results quantified. Thus, a method to convert the results obtained in cpm into units per ml and to express the relationship between unit and nanogram of IgE, will be described. By using the assay procedure as described in III:14, the results are expressed as cpm-values, which are related to the amount of radiolabelled antibody reacted with IgE antibody bound to the immobilized antigen. Thus a cpm-value represents only a proportional measure within the same assay that is dependent upon a number of parameters such as the time of incubation, labelling efficiency of antibody, antibody avidity as well as the amount of antigen conjugated to the paper disc. In the IgE-PRIST, all these factors can be controlled by using standard dilutions of IgE, whereas in PRAST it is necessary to standardize at different levels of IgE antibody in order to compensate for the influence of nonspecific variable factors.

In fig. 15, the dose-response curves obtained by PRAST of 3 different reference sera are compared. For two sera, containing

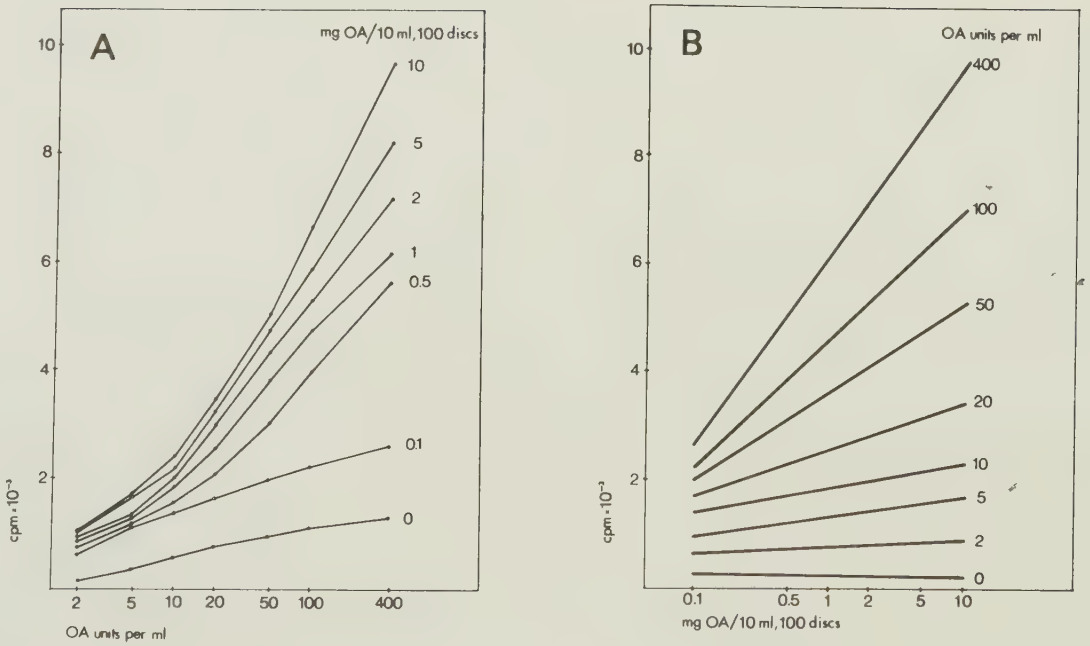


Fig. 16. Uptake of rat IgE antibody as a function of the amount of matrix-bound antigen. Reference serum 1 (HL-anti-OA) in a series of dilutions from 2 to 400 OA-PRAST units per ml was used. Antigen: ovalbumin (OA). Incubation periods: 1st = 6 hrs and 2nd = 15 hrs at RT. Second antibody: ^{125}I-rabbit-anti-rat IgE.

IgE antibody to ovalbumin (OA) and having the same PCA titer but differing in total serum IgE by a factor 17, the standard curves were practically identical. The third curve, parallel to the two mentioned above, was obtained by serial dilutions of a serum containing IgE antibody to N. brasiliensis antigen. The experiments summarized in Fig. 15 thus illustrate, that the shape of the standard curve for PRAST is independent of the specificity of IgE antibody as well as large variations in total IgE in the sample.

For the standardization of PRAST, when IgE antibody directed to different antigens are to be measured, the amount of antigen covalently linked to the paper disc, must also be taken into consideration. Fig. 16 illustrates the uptake of rat IgE antibody to ovalbumin (HL-anti-OA) as a function of the amount of OA bound to the cellulose matrix. For a chemically pure and well defined antigen like ovalbumin, the optimal amount to be used for coating the paper discs can be worked out without too much difficulty, whereas in the case of crude antigens, like N. brasiliensis antigen, the optimal conditions have to be determined for each preparation.

In the present study the following antibody and antigen amounts have been used during the disc coupling procedure (1,000 discs in 40 ml buffer) to obtain discs for standardization: anti-IgE(IR162) - 300 µg immunosorbent purified antibody, ovalbumin - 10 mg, Nippostrongylus brasiliensis antigen - 10 ml (1,000 worm equivalents per ml). To make it possible to compare an IgE antibody response to different antigens or compare the activity of an IgE antibody to different batches of the same antigen, a method for evaluation of the absolute amount of a particular IgE antibody is required. Lundqvist (1975) has described a procedure for the standardization of human IgE, as determined by Phadebas-RAST, by which the RAST-values are calibrated against the PRIST-system used for determination of total IgE. This approach, as it has been applied for the quantitation of rat IgE antibody, is demonstrated in Fig. 17.

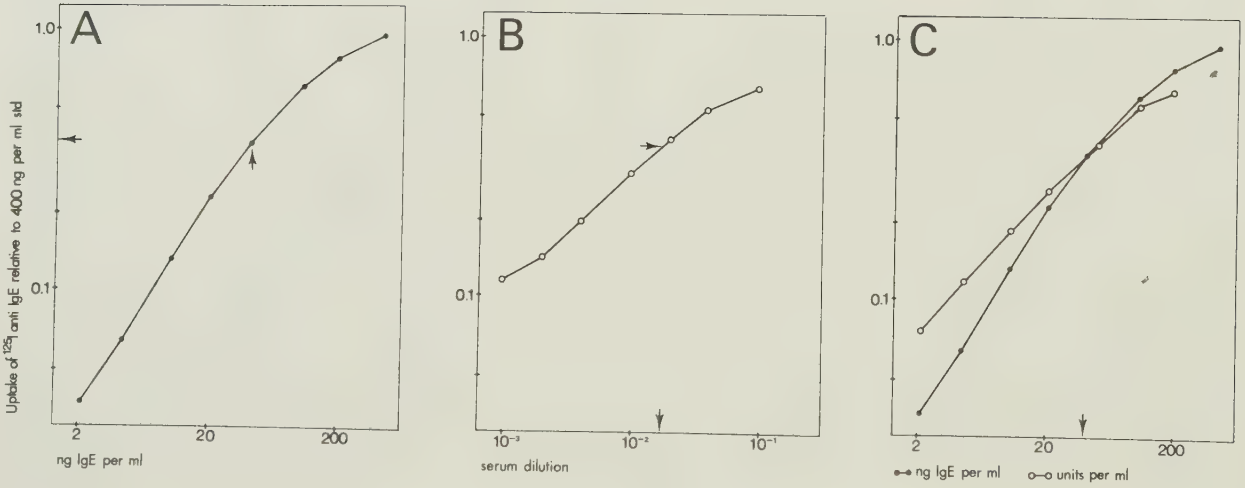

A
1.0
0.1
Uptake of ^{125}I anti IgE relative to 400 ng per ml std
2
20
200
ng IgE per ml
B
1.0
0.1
10^{-3}
10^{-2}
10^{-1}
serum dilution
C
1.0
0.1
2
20
200
ng IgE per ml
units per ml

Fig. 17. Standardization of PRAST for determination of rat IgE antibody by calibration of the assay against a PRIST standard curve for quantitation of serum IgE (Lundkvist, 1975) and using ^{125}I-labelled rabbit anti-rat IgE.

A: PRIST standard curve for total serum IgE. Arrows indicate an arbitrarily selected reference concentration of IgE and the corresponding relative uptake of radiolabelled antibody.

B: PRAST curve for serial dilutions of a reference serum,containing IgE antibody (HL-anti-OA), obtained by using the same experimental conditions as used for evaluation of the PRIST curve in A, above. Arrows indicate the dilution giving the same uptake of radiolabelled antibody as the reference concentration selected in A, above. This dilution, times the corresponding concentration (ng per ml) of IgE, will give the concentration of IgE antibody (in units per ml) in the undiluted serum.

The antibody amount used in A during the coupling procedure was 300 µg per 40 ml buffer to 1,000 CNBr-activated discs and the corresponding amount of ovalbumin in B was 10 mg.

C: PRAST curve for dilutions of the standard serum used in B, expressed in units per ml and compared with PRIST, performed on dilutions of an IgE standard, expressed in ng per ml. The arrow indicates that the IgE antibody concentration (units per ml) at the intersection of the PRIST (●----●) and the PRAST (O----O) curves is numerically equal to the reference concentration (ng per ml) of IgE selected in A above.

After performing PRAST on a series of dilutions of the serum to be evaluated, the dilution which gives the same radioactive uptake as an arbitrarily selected concentration of IgE determined by PRIST is calculated. This dilution, multiplied by the value for the corresponding concentration of IgE expressed in ng per ml, will give the concentration of IgE antibody expressed in units per ml of the undiluted serum. PRAST can now be performed on a second series of dilutions of the same serum, and the results, in units per ml, can be plotted together with the PRIST ng values, assayed under the same conditions, on the same scale. As shown in Fig. 17C the PRIST and PRAST curves intersect at a point which is numerically equal to the reference concentration for IgE that was arbitrarily selected from the PRIST standard curve.

The standardized serum can now be used for PRAST measurements of unknown samples and also for standardization of other sera containing IgE antibody to OA. When standardized sera are used as references, the amount of antigen on the discs can be chosen over a wide range without influencing the assay results. In the experiments shown in Fig. 17, anti-IgE was used as the second, radiolabelled, antibody in both PRIST and PRAST. Since the same antibody was also used for coating the paper discs in PRIST, it can be argued that the IgE molecule in the sample will be oriented or exposed for reaction with second antibody in different ways when determined by PRIST or PRAST. In PRAST, at least one of the antigen binding Fab regions must be bound to the antigen on the disc. Thus, all antigenic determinants within the Fc region are, at least in theory, free to react with radiolabelled antibody, whereas in PRIST, at least one of the Fc determinants must be occupied while remaining antigenic sites might be less accessible to second antibody due to steric effects.

The experiments described in Fig. 17 were therefore repeated using identical conditions, except for the second antibody, which was replaced by rabbit anti-rat Fc-ε. As shown in Fig. 18,

the PRIST and PRAST curves overlap and, as a consequence, the reference concentration on the PRIST standard curve could be selected within a much wider range. Of importance for the use of PRAST standardization for routine measurements of IgE antibody is that the amount of antigen on the paper disc should be constant and that the PRIST and PRAST systems are run under identical conditions.

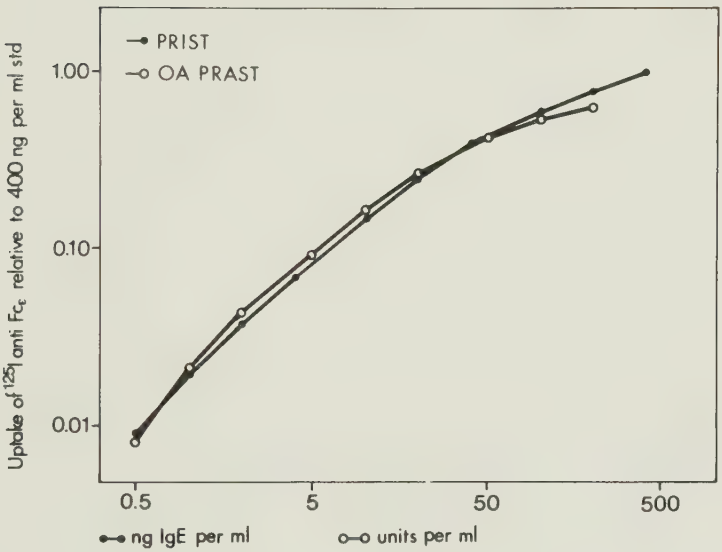


Fig. 18. The effect of using ^{125}I-anti-rat Fc-ε for the standardization of PRAST by calibration against PRIST.

From the discussion above it can be concluded that one PRAST unit of IgE antibody presumably corresponds to less than one ng IgE. This can also be noticed in the immunization experiments (see IV:4) performed in low total IgE rats (Fig. 24), where IgE antibody, expressed in units per ml, increases more than total serum IgE, expressed as ng per ml. Under the conditions used during the standardization, one PRAST unit is approximately equivalent to 1 ng. In a situation where immunologically intact IgE antibody can be isolated, it should be possible to measure the IgE amount in PRIST and PRAST and thereafter calculate the amount of IgE that corresponds to one PRAST unit.

3. The IgE level in serum from apparently healthy nonimmunized rats.

As an early result of the application of PRIST, significant differences in the IgE level in serum from rats of different strains were observed. Sprague Dawley rats, used in the very first immunization experiments, were found to have an extremely low basic serum IgE level as compared to rats of the Hooded Lister strain. A question of major concern is, therefore, whether the IgE level found in a nonimmunized and apparently healthy rat of a particular strain represents a characteristic genetic feature or merely reflects the prevailing conditions of animal housing (breeding, food etc.) or of passing diseases. Furthermore, as there seems to be a strong relationship between the normal IgE level and the levels reached after active immunization, as will be discussed in a following section, a study of the variation of IgE in nonimmunized animals of different age, sex and strains seems warranted.

Variation with age: Rats of Hooded Lister strain have a relatively high basic level of serum IgE and were therefore selected for a study of the variation of IgE with age. Serum from 3-5 rats from different litters was collected at selected days from birth up to 90 days. As shown in Table 1, no IgE was detected until animals were 13 to 14 days old. From day 15, however, the IgE level rapidly increased in an exponential fashion until day 56, when a plateau of 300-500 ng/ml was reached, corresponding to the normal level in nonimmunized Hooded Lister rats. It should be noted that in young adult animals a great variation in the IgE level might be found even in serum from animals of the same litter. With increasing age there is a tendency for less variation as well as a slight decrease in the IgE level, as shown in Table 2, where data for 37 female Hooded Lister rats at 10 weeks and 12 month of age are compared.

TABLE 1

Variation in the serum IgE level with age. Samples were obtained from Hooded Lister rats of both sexes kept under normal breeding conditions. 3 different experiments are combined. 3 - 5 rats were tested per group per day.

Day	Serum IgE, ng per ml
0	0
1	0
6	0
7	0
13	+
14	+
15	5
20	25
40	125
56	315
90	500

TABLE 2

The serum IgE level in 37 female Hooded Lister rats at 10 weeks and 12 months.

	10 weeks	12 months
IgE, ng per ml, range	54-1900	40-2240
" mean	549	405
" S.E.M.	73	68
Distribution	%	%
0- 500 ng per ml	59	75
501-1000 "	27	19
1001-1500 "	11	3
1501- "	3	3

TABLE 3

Total IgE in serum from male and female Hooded Lister rats at the age of 10 weeks. 9 different litters.

Sex	Males	Females
Number of animals	38	49
IgE, ng per ml, range	15-8000	25-6000
" , mean	944	829
" , S.E.M.	270	142
Distribution	%	%
0- 500	66	49
501-1000	11	21
1001-1500	5	14
1501-	18	16

Sex differences: To determine if there are any sex differences with respect to the IgE level, sera from 38 male and 49 female 10 week old Hooded Lister rats from 9 litters were investigated. As shown in Table 3, no significant differences in the IgE level for the two groups were found. The same conclusion was reached after examination of the results presented in Tables 4 and 6, which summarize the IgE level in female and male rats of different strains kept under different breeding conditions. One obvious problem in comparing female and male rats is that the animals cannot be kept in the same cage for an extended time and, therefore, the living conditions are not exactly the same.

TABLE 4

Total IgE in serum from various rat strains kept by Dr. H. Bazin, University of Louvain, Brussels. Five rats of each sex. The age of animals from different strains varied from 4 to 12 months, but animals from the same strain were of the same age.

Strain	Sex	Total IgE in serum, ng/ml		
		Range	Mean	
FISCHER	F	0-75	24	1436
	M	937-6982	2847	
OKAMOTO	F	35-1047	465	542
	M	217-1288	618	
AxC	F	5-1698	657	637
	M	185-1365	616	
DA	F	317-1799	772	707
	M	71-1479	641	
LOU/CIg4(OKA)	F	565-7312	2172	2418
	M	81-8892	2664	
LOU/M	F	504-2094	1502	1288
	M	83-2576	1073	
LOU/C	F	1485-3475	2149	2795
	M	1803-5610	3440	

TABLE 5

IgE in sera from nonimmunized rats in 5 different laboratories (A - F). Each group contained 4 - 15 animals with ages from 3 to 5 months.

Strain	Lab.	IgE, ng per ml range	mean
AS	B	0-2	1
AUGUST	E	25-62	44
BN 2)	B	200-1120	660
BN	F	2030-4100	2850
DA	F	322-863	520
FISCHER	E	3-15	10
FISCHER	B	35-98	67
HL 1)	A	15-8000	841
HL	B	520-650	585
HL	F	3520-8250	4300
LEWIS 2)	E	250-1040	452
LEWIS	E	17-44	32
LOU	D	2200-14800	8150
LOU	A	23-1000	365
SD	A	0-24	5
SD 2)	A	0-45	12
SD	C	3-28	11
WISTAR	B	9-22	16
WISTAR	E	100-2050	563

1) 147 rats 2) 12 months

TABLE 6

The serum IgE level in 6 week old rats of different strains kept under conventional or SPF breeding conditions in Møllegaards Avlslaboratorium ApS, L. Skensved, Denmark.

Strain	Sex	Number	Breeding conditions	IgE, ng/ml Mean		Range
SD	F	5	conv.	1	24	0-4
SD	M	5	conv.	47		8-70
SD	F	5	SPF	0	0	0-1
SD	M	5	SPF	0		0-1
WISTAR	F	5	conv.	43	34	0-186
WISTAR	M	5	conv.	25		4-61
WISTAR	F	5	SPF	0	0	0-1
WISTAR	M	5	SPF	2		0-7
LEWIS	F	5	conv.	0	1	0-1
LEWIS	M	5	conv.	0		0-1
HL	F	5	conv.	42	67	27-60
HL	M	5	conv.	91		31-200
SHR	F	5	conv.	11	6	2-92
SHR	M	5	conv.	0		0-3

Effect of breeding conditions: One early result of using the IgE-PRIST technique on rat serum obtained from different laboratories was the observation that even rats of the same strain may show a very great variation in IgE, as shown in Tables 3 and 4. The high levels in Hooded Lister, Brown Norway, Lewis and Wistar rats in Table 5 compared to the normal level of these 4 strains, shown in Tables 6 and 7, is probably expression of different breeding and living conditions. Another example is the LOU strain. In samples obtained from LOU rats kept by Dr. H. Bazin, University of Louvain, the average level was 4-10 times the level found in the LOU/C and LOU/M rats bred in our own animal department but originating from Dr. Bazin's LOU rats one or two generations earlier. Fischer rats have normally been found to have a low basic level of IgE (usually less than 50 ng per ml). Therefore, the great difference found between the two groups of female and male Fischer rats kept by the same breeder is noteworthy (Table 4). That different breeding conditions may influence the results is demonstrated in Table 6, where IgE data for 6 week old rats from 5 strains kept under conventional and SPF breeding conditions are compared. The extremely low IgE level in SPF bred animals indicates that infections cannot be excluded as one cause of variation in the IgE level found in conventionally bred rats. That parasitic infestations will stimulate an increased synthesis of IgE is well documented (Jarret and Bazin, 1974) and therefore an undiagnosed infection should be suspected whenever a great deviation from the normal IgE level is observed.

Variation with strain: To determine the impact of genetic factors on the IgE level, hybridization experiments were performed using animals from strains normally having low and high IgE levels. Rats of the strains AS(low IgE), Fischer(medium IgE), Brown Norway(high IgE) and Hooded Lister(high IgE) were cross-mated to give 6 different types of F1 hybrids (Table 7). The animals were from the same breeding stock and two hybridization experiments were done at different occasions. Sera from

TABLE 7

The total serum IgE level in 4 rat strains and their F1 hybrids obtained in two different experiments (A and B) with 3 - 4 rats per group.

Strain	Serum IgE, ng/ml		Mean
	A	B	
AS	1	2	2
FISCHER	67	25	46
BROWN NORWAY	660	177	419
HOODED LISTER	585	560	573
FI/AS	40	20	30
HL/AS	289	238	264
FI/HL	210	325	268
FI/BN	450	175	312
BN/AS	259	630	445
HL/BN	952	1435	1194

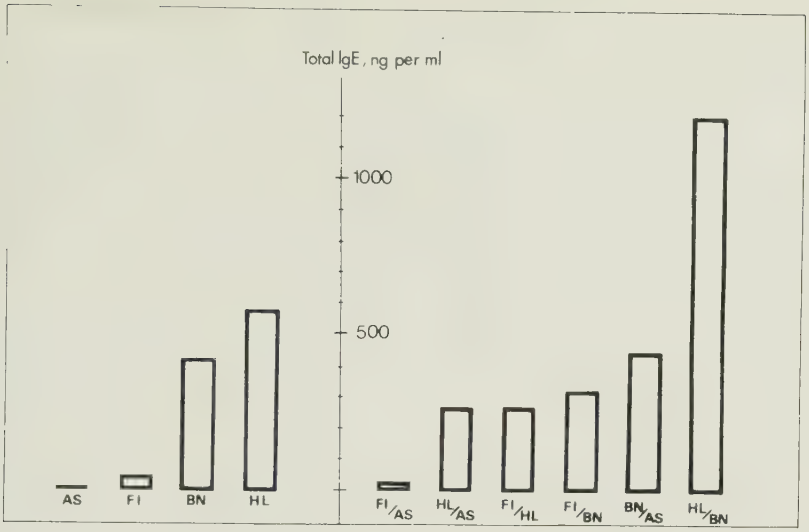


Fig. 19. The serum IgE level in adult (3-5 months) rats of 4 different strains and their Fl hybrids, as determined by PRIST. (For details see Table 7).

TABLE 8

The serum IgE level in parents and Fl hybrids of Hooded Lister and Sprague-Dawley rats.

Sex	Parent	IgE, ng/ml	F_1	IgE, ng per ml Range	Mean	Mean F+M
F	HL	1667	4	6-312	94	109
M	SD	8	8	15-400	117	
F	SD	19	8	23-400	169	136
M	HL	1750	4	18-108	70	
F	HL	52	5	8-23	16	12
M	SD	8	8	0-25	9	
F	SD	20	6	4-45	20	15
M	HL	21	5	6-16	10	

three randomly selected rats in each group were analyzed for IgE by PRIST. As shown in Table 7 and Fig. 19, an increased IgE level was found in F1 hybrids when both parents came from strains which normally have high IgE levels, whereas F1 hybrids having medium IgE were obtained when only one of the parent strains had high IgE. Hybridization of Sprague Dawley and Hooded Lister rats, which were the two strains used in most of the immunization experiments described in this thesis, confirmed the above results. To determine if there might be a linkage between the IgE level in the hybrid and the sex of the parent having a high IgE, both female and male SD rats were used in combination with female and male HL rats. As shown in Table 8, the IgE level in F1 hybrids seems to be influenced by the IgE level of the HL parent. Thus, using a HL parent with high IgE resulted in F1 hybrids having a significantly higher IgE level as compared to hybrids derived from a HL parent having a low IgE level. However, in neither case did the IgE level in the hybrids reach the level characteristic of the HL parent. The effect of back-crosses and brother-sister matings are shown in Table 9. To conclude, all these results indicate that IgE production is under the control of several genes, of which those confined to the suppression of IgE synthesis or release appear to play a dominant role. No correlation between the IgE levels and AgB type can be concluded from the present studies because of the small number of strains tested (Table 10).

Although the Hooded Lister strain is characterized by a high IgE level, great variations can be found for rats in the same litter. Since genetic rather than environmental factors are likely to explain the observed variation, the effect of repeated inbreeding of HL rats selected for either high or low IgE production was performed. As shown in Table 11, selected inbreeding of HL rats with high IgE will result in normal to high IgE levels in the offspring, whereas selection for parents with low IgE will result in normal IgE levels in the offspring.

TABLE 9

The serum IgE level in various hybrids of Hooded Lister and Sprague Dawley rats. The effect of backcross and brother-sister matings.

Sex	Parent	Type	IgE, ng/ml	F_1	HL-type,%	IgE, ng per ml Range	Mean	Mean(F+M)
F	SD	-	1	7	29	0-3	1	2
M	SD/HL	HL	0	8	13	0-12	3	
F	SD/HL	HL	4	5	80	12-340	121	101
M	SD	-	10	8	63	8-560	88	
F	HL/SD	HL	12	7	100	40-144	74	74
M	HL	-	4000	6	100	46-104	74	
F	HL/SD	HL	7	1	100	58	58	122
M	HL	-	4000	10	100	44-260	128	
F	SD/HL	HL	108	7	71	3-74	30	36
M	SD/HL	HL	0	5	100	1-185	44	
F	SD/HL	HL	31	6	50	0-5	1	0
M	SD/HL	HL	67	4	50	0-1	0	
F	SD/HL//HL	HL	4000	3	100	32-120	67	84
M	SD/HL//HL	HL	4000	4	100	36-182	96	
F	SD/HL//SD	HL	340	8	75	0	0	0
M	SD/HL//SD	HL	560	3	67	0	0	
F	SD/HL//SD	HL	27	3	100	0	0	4
M	SD/HL//SD	HL	8	4	50	0-30	8	

TABLE 10

The relative serum IgE levels in nonimmunized rats.

AgB type	Rat strain	IgE levels (ng per ml) LOW (0-50)	MEDIUM (50-300)	HIGH (>300)
1	FISCHER	X	(X)	
	LEWIS	X	(X)	
	AS	X		
2	WISTAR	(X)	X	
	LOU			X
3	BN			X
4	DA			X
5	AUGUST		X	
	HL			X
6	SD	X		

TABLE 11

The effect of selective inbreeding of Hooded Lister rats having high (A), low (B) and normal (C) serum IgE levels

	Generation of parents	Sex	IgE, ng/ml	F_1	IgE, ng per ml Range	Mean	Mean (F + M)
A.	1	F	480	5	500-6000	2120	1816
		M	-	3	400-1620	1310	
	2	F	2200	4	>4000	>4000	>4000
		M	1620	2	>4000	>4000	
	3	F	>4000	5	138-198	162	168
		M	>4000	5	88-312	173	
	4	F	(162)	6	124-3500	1947	2244
		M	(173)	7	850-8600	2499	
B.	1	F	150	8	25-220	49	48
		M	-	7	15-88	47	
	2	F	29	6	5-59	35	26
		M	15	3	0-13	9	
	3A	F	15	13	42-840	265	255
		M	0	5	110-560	229	
	3B	F	5	4	114-220	173	174
		M	0	7	120-268	175	
	4B	F	(173)	3	460-1300	740	630
		M	(175)	4	126-820	547	
C.	1	F	21	9	238-1125	584	597
		M	-	4	134-1250	627	
	2A	F	1025	6	290-680	536	536
		M	1250	5	205-765	536	
	3A	F (3)	(536)	15	90-1083	676	614
		M (3)	(536)	14	207-1140	548	
	2B	F	1125	4	178-710	435	446
		M	1250	3	178-635	461	
	3B	F (3)	(435)	7	199-1354	682	791
		M (3)	(461)	12	209-2880	854	

4. The IgE response in immunized rats.

In the present section, the variation in total IgE and specific IgE antibody after immunization of Hooded Lister and Sprague Dawley rats is studied using PRIST and PRAST. Infection by helminths potentiates the IgE response in man and in rodents (Johansson et al., 1968b; Orr and Blair, 1969). In the rat, the effect of infection differs with the strain. In Hooded Lister rats infected with Nippostrongylus brasiliensis larvae the IgE level may rise to more than 100 µg per ml (Jarrett and Bazin, 1974), whereas similarly infected Sprague Dawley rats reach a level of total serum IgE of not more than 10-15 µg per ml. Similar differences in the IgE response between strains normally having high or low basic IgE levels are also obtained after immunization with soluble antigen in the presence of adjuvant.

Total IgE and IgE antibody after infection with N. brasiliensis: To obtain high levels of IgE, 2,000-4,000 N. brasiliensis larvae were injected s.c. The life cycle of N. brasiliensis is illustrated in Fig. 20. For studies of the effect of simultaneous infection and immunization, 1-100 µg of antigen were given by intraperitoneal injection.

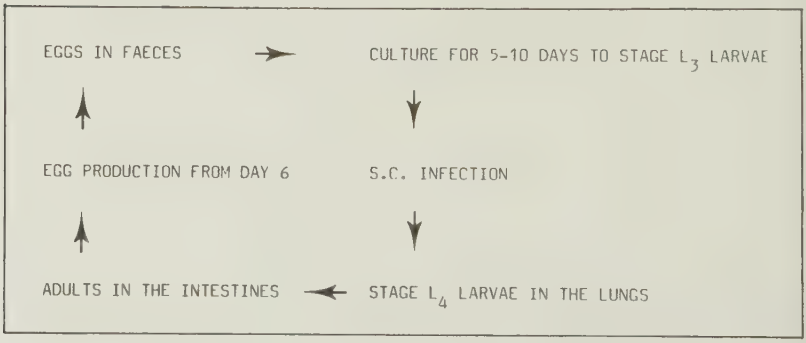
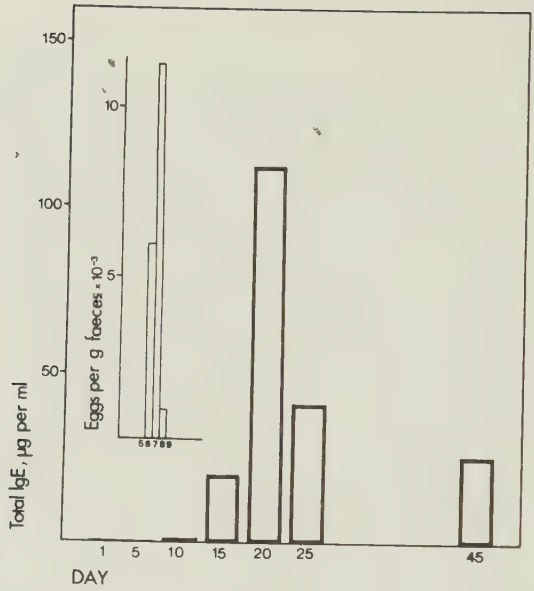

EGGS IN FAECES
CULTURE FOR 5-10 DAYS TO STAGE L_3 LARVAE
EGG PRODUCTION FROM DAY 6
S.C. INFECTION
ADULTS IN THE INTESTINES
STAGE L_4 LARVAE IN THE LUNGS

Fig. 20. Life cycle of Nippostrongylus brasiliensis.

The results from 3 experiments using Hooded Lister rats infected with parasite are summarized in Fig. 21. The onset of IgE synthesis takes place during development of the parasite, but the maximum IgE level is not reached until the parasites have been expelled i.e., when the egg production is discontinued. In all 3 experiments, a peak level of IgE amounting to 80-130 µg per ml and corresponding to a 500-fold increase over the normal IgE level was reached on day 20. After day 20, gradual but slow decrease in serum IgE was observed and more than 2 months after infection the IgE level was still found to be about 40 times the preinfection level. By taking into consideration the short half-life of circulating IgE, which in the rat has been reported to be 1-2 days (Tada et al., 1975), the slow decrease in serum IgE illustrates the profound effect of parasitic infection on regulatory mechanisms for IgE synthesis and secretion.

The differences in total serum IgE and IgE antibody between Hooded Lister and Sprague Dawley rats after infection with N. brasiliensis larvae are shown in Fig. 22. The levels of serum IgE and IgE antibody in Hooded Lister rats were 10-15 times higher than the levels found in Sprague Dawley rats, and this difference between the two strains was practically constant during the course of the experiment. It should be noted that the distribution of IgE antibody to N.brasiliensis antigen does not coincide with the distribution of total serum IgE. Thus, antibody production does not approach its maximum level until about 2 weeks after the maximal production of total IgE, indicating that parasitic antigens are not released until about 2-3 weeks after infection.

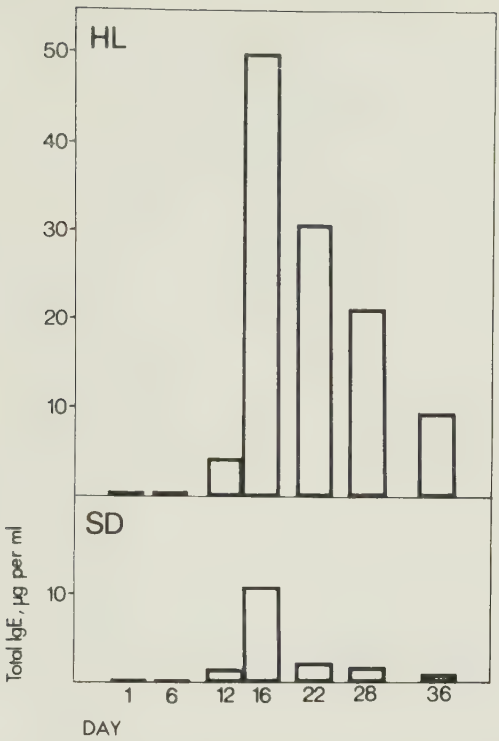
Secondary IgE response in parasite infected rats: To determine the effect on the secondary IgE response, 3 groups of infected Hooded Lister rats were subjected to, respectively, re-infection with N.brasiliensis, immunization with N.brasiliensis antigen and immunization with B.pertussis vaccine. The results are summarized in Table 12. In experiment A, 8 rats were infected with 2,000 N.brasiliensis larvae per animal and bled once a week during the first month. After 6 months, the rats were



Day	IgE, ng per ml A	B	C	Mean
1	100	490	116	235
5	56	392	71	173
10	444	1100	595	713
15	16600	16000	18300	19967
20	124000	134000	81600	113200
25	51600	35700	34900	40733
45	28900	20400	28400	25900

Day	Eggs per g faeces A	B	C	Mean
5	0	0	0	0
6	423	3990	13098	5837
7	7354	14186	12468	11336
8	-	418	1490	954
9	0	0	0	0

Fig. 21. Total serum IgE as determined by PRIST, and number of helminth eggs in faeces, in Hooded Lister rats after infection with Nippostrongylus brasiliensis (N.b.) larvae. 3 groups (A-C) of 3 rats each, were injected s.c. on Day 1 with 4.000 larvae per animal.



Day	Total IgE, ng per ml HL	SD	HL/SD	IgE antibody, cpm (N.b. PRAST) HL	SD	HL/SD
1	305	0	-	0	0	-
6	325	3	108	44	0	-
12	4110	1002	4	157	17	9
16	50000	10605	5	426	35	12
22	30800	1808	17	507	139	4
28	21600	1451	15	1066	95	11
36	9400	477	20	1632	132	12

Fig. 22. Total IgE and IgE antibody in serum from Hooded Lister and Sprague Dawley rats infected with N. brasiliensis larvae (4000 larvae s.c. per animal). Four rats in each group.

TABLE 12

Total IgE and specific IgE antibody in Hooded Lister rats after primary infection with 2000 N.b. larvae s.c. per animal and

A) N.b. antigen immunization, 100 µl i.p per rat

B) Reinfection with N.b., 500 larvae s.c. per rat

C) B. pertussis immunization, 10^{10} i.p. per rat

Treatment	Day[a)]	Total IgE µg/ml	IgE antibody to N.brasiliensis units/ml	Total IgE / IgE antibody
A. Primary infection	1	-	-	-
	6	2.12	0	-
	13	30.00	16.4	1829
	18	45.50	25.9	1757
	25	20.85	48.0	434
	31	18.75	136.5	137
Immunization	180 (1)	0.34	0	-
	185 (6)	0.76	24.3	31
B. Primary infection	1	-	-	-
	15	168.4	38.3	4396
Secondary infection	41 (1)	17.6	51.0	344
	46 (6)	144.4	113.8	1268
	51 (11)	101.6	102.5	991
	56 (16)	63.4	59.8	1059
	61 (21)	30.5	56.8	537
C. Primary infection	1	0.1	0[b)]	-
	5	0.06	0	-
	10	0.44	46	-
	15	16.6	370	-
	20	124.0	4229	-
	25	51.6	4688	-
B. pertussis	45 (1)	28.9	7376	-
	52 (8)	31.5	6510	-
	60 (16)	12.8	5898	-

a) (1) day of secondary immunization (infection). b) PRAST in cpm.

bled and given 100 µl of N.brasiliensis antigen (corresponding to 1,000 worm equivalents per ml) per animal by i.p. injection, and after 5 days bled again. In experiment B, 20 rats were re-infected with 500 N.brasiliensis larvae per animal by s.c. injection on day 41 after the primary infection. The animals were bled every fifth day. In experiment C, 4 rats were given a primary infection with 2,000 N.brasiliensis larvae by s.c. injection. Serum was collected at every fifth day until day 25. On day 45 the animals were bled and given 10^{10} B.pertussis by i.p. injection. Serum was collected on day 7 and 14 after immunization. In contrast to the results obtained after primary infection, re-infection produced a rapid and simultaneous response in both total serum IgE and IgE antibody (Fig. 23). On day 6 the serum IgE level was 8 times higher and the IgE antibody level 2 times higher than on the day of re-infection. Administration of B.pertussis vaccine was observed to have little effect on the IgE response, whereas immunization with N.brasiliensis antigen, 6 months after primary infection with parasite, resulted in a rapid IgE antibody response (from 0 to 24 units per ml in 6 days) and a 2-fold increase in the total IgE.

Effect of infection with N.brasiliensis on the IgE and IgG response to a nonparasite antigen: Table 13 summarizes the results obtained when Hooded Lister rats were infected with N.brasiliensis larvae and simultaneously immunized with ovalbumin. Four groups of 5 rats were infected on day 1 with 4,000 larvae injected s.c., and simultaneously, the animals in groups I and II received an intraperitoneal injection of 100 µg of ovalbumin in PBS. On day 40, all the animals were re-infected with 500 N.brasiliensis larvae given s.c., and in addition, animals in groups I and III received an injection of 100 µg of ovalbumin given i.p. The results, presented in Table 13, show that with primary immunization with ovalbumin done in conjunction with primary infection with parasite (groups I and II), a weak IgE but no IgG response was obtained,

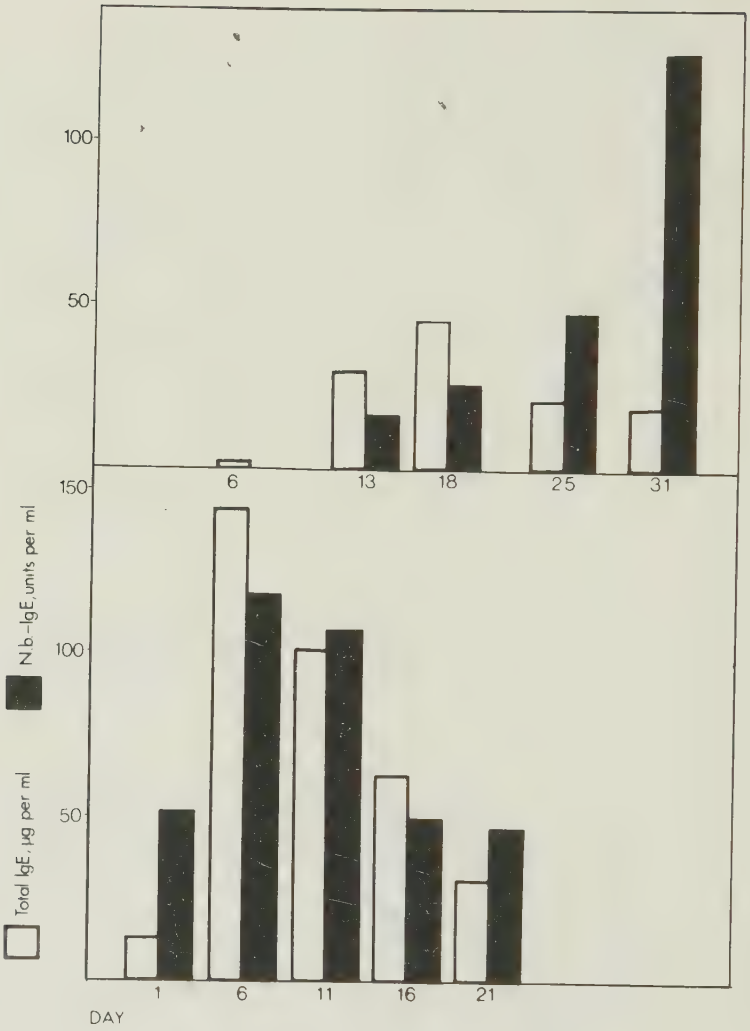


Fig. 23. Total IgE and IgE antibody in serum from Hooded Lister rats after primary (Top) and secondary (Bottom) infections with N. brasiliensis. Primary infection (Day 1) by 2,000 larvae s.c. per animal. Secondary infection (given 45 days after the first) by 500 larvae s.c. per animal.

TABLE 13

The immune response in Hooded Lister rats, infected with N. brasiliensis and simultaneously given soluble antigen, as determined by PRIST (total IgE) and by PRAST (IgE and IgG antibody). Four groups of 5 rats were injected (day 1) with 4.000 N. brasiliensis larvae, s.c. (groups I-IV) and 100 µg of ovalbumin (OA), i.p. (groups I, II) and re-injected (day 40) with 500 N.br. larvae, s.c. (groups I-IV) and 100 µg of OA, i.p. (groups I, III).

Day	IgE antibody, units/ml							
	N.br.-antigen				OA-antigen			
	I	II	III	IV	I	II	III	IV
15	19	61	37	36	1	0	0	0
41	38	63	61	42	0	0	0	0
46	85	131	138	101	0	0	0	0
51	54	133	103	120	6	0	4	0
56	46	56	68	69	7	2	3	0
61	34	49	58	86	3	2	2	0
	IgG antibody, cpm							
15	3438	0	0	0	0	0	0	0
41	1050	1823	227	1328	0	0	0	0
46	2613	772	4789	2643	4858	507	570	0
51	0	2034	1078	1659	6942	491	0	0
56	0	2360	1041	2709	4994	0	0	0
61	281	2384	2475	1798	8541	74	772	0

Day	Total IgE, µg per ml			
	I	II	III	IV
15	87	192	162	233
41	15	15	22	18
46	99	140	140	199
51	66	91	146	104
56	31	43	77	108
61	18	18	42	45

as measured up to day 40 by PRAST, in contrast to the normal development of an IgE response to N.brasiliensis antigen during the same period. After administration of a second dose of antigen in conjunction with re-infection (group I) a strong secondary response of both IgE and IgG antibody to ovalbumin was obtained. A secondary IgE/IgG response to antigen, but of smaller magnitude, was also produced by re-infection in the absence of antigen (group II). This result is in agreement with the finding of Jarrett and Stewart (1972) that parasitic infections of previously antigen primed rats will potentiate an IgE response to the antigen. In this context it is of interest to note that immunization of previously infected animals with a nonparasite antigen (group III) will also potentiate the production of both serum IgE and IgG antibody.

The early IgE antibody response: To study the early IgE response, two groups of Hooded Lister rats were compared after infection with N.brasiliensis larvae or immunization with soluble antigen in the presence of B.pertussis vaccine. One group of 8 animals were given 1 µg of ovalbumin by i.p. injection together with 10^{10} B.pertussis and a second group of 8 animals were infected with 4,000 N.brasiliensis larvae by s.c. injection. On day 12, the animals in the first group were given a second injection i.p. of 1 µg of ovalbumin without adjuvant. Serum was collected every second day and analyzed for total IgE and IgE antibody. The results are summarized in Table 14. A positive IgE antibody response to ovalbumin was obtained 8 days after immunization, as determined with both IgE-PRAST and PCA, whereas no IgE antibody to parasitic antigens could be detected by either method until 12 days after infection. These results are in concert with the data presented in Fig. 21 and Table 12. As IgE antibody to N.brasiliensis is determined by using adult worm antigen, the results indicate that this antigen will not be produced and secreted until the parasites have migrated into the intestinal tract (Jarrett and Stewart, 1973 ; Ogilvie and Hockley, 1968).

TABLE 14

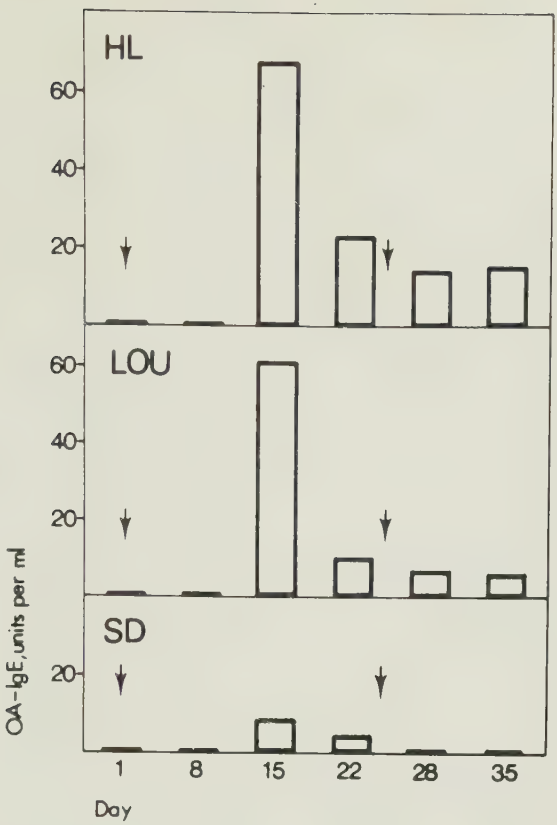
The early IgE response in Hooded Lister rats immunized with ovalbumin (OA) and B. pertussis (1 µg OA and 10^{10} B.p. day 1 and 1 µg OA day 12) or infected with N. brasiliensis larvae (4,000 larvae s.c. on day 1), as determined by PRIST (total IgE), PRAST (IgE antibody) and PCA (IgE antibody).

Day	Ovalbumin - B. pertussis			N. brasiliensis		
	Total IgE µg/ml	IgE antibody to OA cpm	PCA[a] titer	Total IgE µg/ml	IgE antibody to N. brasiliensis cpm	PCA[a] titer
2	0.33	0	0	0.67	0	0
4	0.33	0	0	-	-	-
6	0.31	0	0	-	-	-
8	0.30	170	1	17	0	0
10	0.32	970	4-8	-	-	-
12	0.36	640	4	130	380	0
16	0.40	3060	256	110	670	64

a) reciprocal dilutions

The effect of Freund's complete adjuvant (FCA): Experimentally induced IgE responses in animals depend upon the type of adjuvant used in immunization. Both aluminium hydroxide gel and B.pertussis vaccine have been found to be effective (reviewed by Tada, 1975). Though they represent the two types of adjuvants most frequently used in conjunction with studies of the IgE response, inconsistent results are occasionally obtained due to variations in quality. With respect to B.pertussis, the improved methods now commonly used by most vaccine manufacturers seem to yield a product having little if any adjuvant effect. To study if FCA, which is commonly available and of constant quality, might be used as adjuvant, its effect on the IgE response in Hooded Lister, LOU and Sprague Dawley rats was determined. Three, 8 week old rats from each strain were given s.c. injections of 100 µl FCA mixed with 100 µg of ovalbumin in 100 µl PBS. The animals were bled once a week until day 24, when a second injection of adjuvant (100 µl FCA in 100 µl PBS) was given s.c. Total serum IgE and IgE antibody to ovalbumin were determined by PRIST and PRAST and the results are shown in Fig. 24. The maximal IgE antibody response obtained in Hooded Lister and LOU rats was of similar magnitude, the only significant difference noted between these two strains being a more rapid decrease in IgE antibody in LOU rats. As could be expected, a less pronounced IgE response was obtained in Sprague Dawley rats with the amount of IgE antibody to ovalbumin corresponding to about 10 per cent of that found in the other two strains. No booster effect resulted from the second injection of adjuvant. With respect to the variation in total serum IgE during the course of immunization, only marginal effects were observed in all three strains.

The effect of antigen dosages in the presence of FCA: The experimental induction of IgE antibody responses in the rat has been reported to be inversely proportional to the amount of antigen used in immunization, within the range of 1 µg to 1 mg of antigen in the presence of B.pertussis adjuvant (Jarrett and Stewart, 1974; Jarrett et al., 1976). To determine the



Day	Total IgE ng per ml			IgE antibody OA units per ml			Total IgE / IgE antibody		
	HL	SD	LOU	HL	SD	LOU	HL	SD	LOU
1	597	5	231	0	0	0	-	-	-
8	905	7	242	0	0	0	-	-	-
15	975	9	213	68	8	61	14	1	4
22	1300	5	264	23	4	10	57	1	26
28	705	3	268	14	0	7	50	-	38
35	880	2	244	15	0	5	59	-	49

Fig. 24. Total IgE and IgE antibody in serum from Hooded Lister, LOU/C and Sprague Dawley rats after immunization with ovalbumin (OA) in Freund's complete adjuvant (FCA). 5 rats of each strain; 100 µg OA in 100 µl FCA were injected s.c. per animal on Day 1. On Day 24, all animals received 100 µl PBS-100 µl FCA, injected s.c.

effect of antigen dosage in the presence of FCA on the immune response with respect to production of IgE and IgG antibody, three groups of 5 adult male Hooded Lister rats were immunized with 10, 100 or 500 µg of ovalbumin, injected s.c. together with 100 µl of FCA. On day 29, all the animals received a second injection s.c. of 10 µg of ovalbumin in 100 µl of FCA. The animals were bled once a week and serum IgE and IgG antibody to ovalbumin were determined by PRAST. As shown in Fig. 25 and Table 15, only a marginal difference was observed in the IgE response between animals immunized with 10 or 100 µg of ovalbumin, with the amounts of antibody being about 3 times as high as that produced by the animals that had received 500 µg of antigen. Though these results are in disagreement with the findings of an inverse relationship between IgE production and antigen dosage, a second injection of a low dose of ovalbumin was found to produce a secondary IgE response only in those animals that had initially received a low amount of antigen. With respect to the effects of antigen dosage on the IgG response, a maximal production of IgG antibody was obtained in those animals that had been given 100 µg of antigen. By injection of a second low dose of antigen, a secondary IgG response was obtained in all the animals. Comparison of the IgE and IgG responses demonstrated that while the level of IgE antibody in serum will reach a distinct peak value within 12-14 days after primary immunization, the maximal IgG antibody response is not reached until 3 weeks after the primary injection of at least 100 or 500 µg of antigen.

The effect of aluminium hydroxide gel: In preliminary experiments investigating the suppression of the secondary IgE response by chemically altered allergen, another immunization protocol was tried using aluminium hydroxide gel as adjuvant (Levine and Vaz, 1970). Five Hooded Lister rats were given 10 µg of dog albumin absorbed to 10 mg of aluminium hydroxide gel and injected i.p. on day 1 and day 23. Total serum IgE

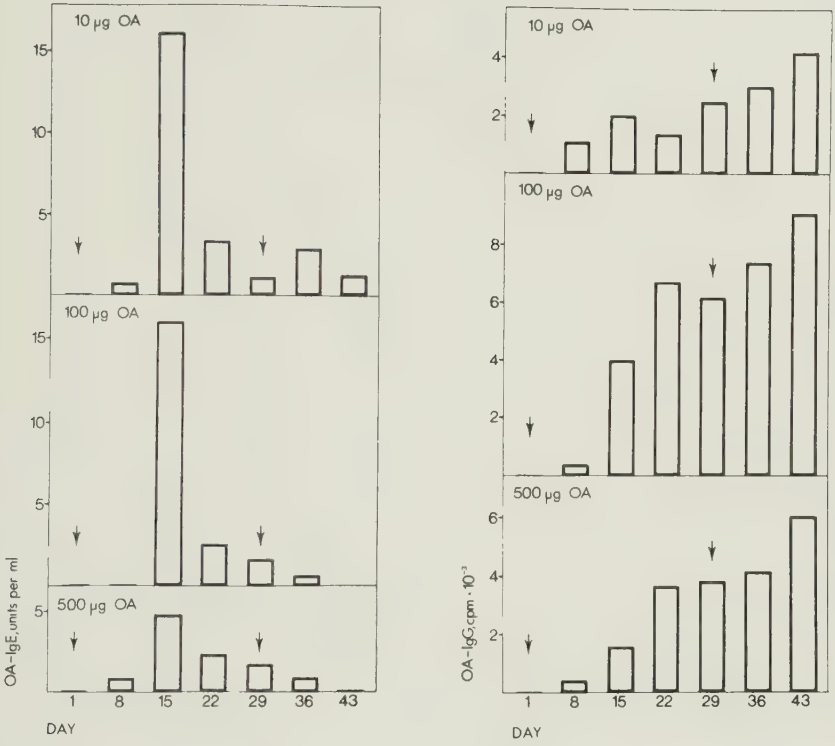


Fig. 25. The IgE and IgG antibody response in 3 groups of 5 Hooded Lister rats, immunized s.c. on Day 1 with 10, 100 and 500 µg of ovalbumin (OA), respectively, in 100 µl FCA. On Day 29, all animals received 10 µg OA in 100 µl FCA by s.c. injections.

TABLE 15

The effect of antigen amount on the immune response in Hooded Lister rats, as determined by PRIST (total IgE) and PRAST (specific IgE and IgG antibody). Three groups of 5 rats were injected s.c. on day 1 with, respectively, 10, 100 and 500 µg of ovalbumin (OA) in 100 µl FCA. On day 29, all animals were injected s.c. with 10 µg OA in 100 µl FCA.

Assay	Day	Amount of antigen					
		10 µg		100 µg		500 µg	
Total IgE	1	688		517		500	
ng/ml	8	665		556		975	
	15	546		423		564	
	22	900		337		627	
IgE antibody	1	0	(0)	0	(0)	0	(0)
units/ml	8	0.7	(0.04)	0	(0)	0.8	(0.05)
(relative	15	16.1	(0.99)	16.2	(1.0)	4.6	(0.28)
values)	22	3.3	(0.20)	2.5	(0.15)	2.2	(0.14)
	29	1.1	(0.07)	1.6	(0.10)	1.6	(0.10)
	36	2.9	(0.18)	0.6	(0.04)	0.8	(0.05)
	43	1.2	(0.07)	0	(0)	0	(0)
IgG antibody	1	0	(0)	0	(0)	0	(0)
Cpm	8	1122	(0.12)	359	(0.04)	412	(0.05)
(relative	15	2038	(0.22)	4036	(0.44)	1595	(0.17)
values)	22	1393	(0.15)	6790	(0.74)	3705	(0.40)
	29	2525	(0.27)	6276	(0.68)	3866	(0.42)
	36	3097	(0.34)	7478	(0.81)	4197	(0.45)
	43	4309	(0.47)	9244	(1.0)	6164	(0.67)

determined by PRIST and IgE antibody was evaluated by PRAST and by PCA. As shown in Fig. 26, the IgE response developed in a fashion similar to that obtained by using either B.pertussis vaccine (see Table 14) or FCA (Fig. 25 and Table 15) as adjuvants, with the exception that the maximal level of antibody was reached 4-5 days later, both after primary and after secondary immunizations. In comparison to the results obtained with B.pertussis (Table 14), potentiation of the IgE response in Hooded Lister rats by using aluminium hydroxide gel appeared less efficient. Fig. 27 illustrates the results obtained by repeated booster injections of antigen adsorbed onto aluminium hydroxide gel. After injection of a booster dose of antigen on day 29 which resulted in a 4-fold increase in IgE antibody, as determined by PRAST, the antibody values decreased quite slowly and more than a month after injection still had not fallen to the maximal level obtained with the primary immunization. By giving a second booster dose of antigen on day 71, a positive but weak tertiary IgE response was obtained.

Comparison of PCA and PRAST for evaluation of the IgE response: To determine the relationship between the values of IgE antibody by PCA and by PRAST three strains of rats (Hooded Lister, LOU/C and Sprague Dawley) were used for PCA determinations on two reference sera, one containing IgE antibody to ovalbumin and the other to N.brasiliensis. Both sera were tested in each animal and all rats were given intracutaneous injections of the same dilutions of serum and challenged 48 hrs after the primary injection with the same preparations of ovalbumin and N.brasiliensis antigen. As shown in Table 16, the PCA titer obtained for a particular serum was dependent upon the strain of rat and the age of the recipient animal. The difference in PCA titers obtained when determined in animals of the same strain and of the same age, amounted to 1-2 titer steps in the case of IgE anti-ovalbumin and 1-3 titer steps in the case of IgE anti-parasite antigen. Although higher PCA titers for IgE antibody to ovalbumin were obtained by using old Hooded Lister, LOU/C and Sprague Dawley rats as compared to the titers obtained in

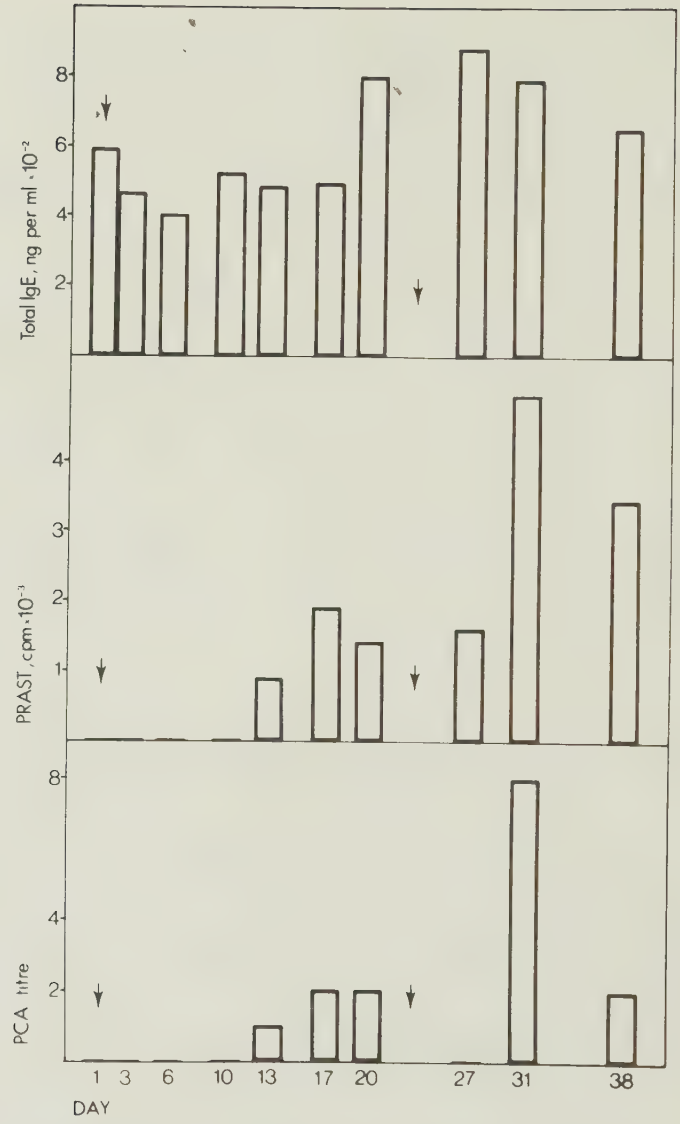


Fig. 26. Total IgE as determined by PRIST, and IgE antibody as determined by PRAST and PCA, in serum from 5 Hooded Lister rats immunized with dog albumin in aluminium hydroxide gel. 10 µg of antigen adsorbed to 10 mg of adjuvant were given by i.p. injection on Day 1 and Day 23.

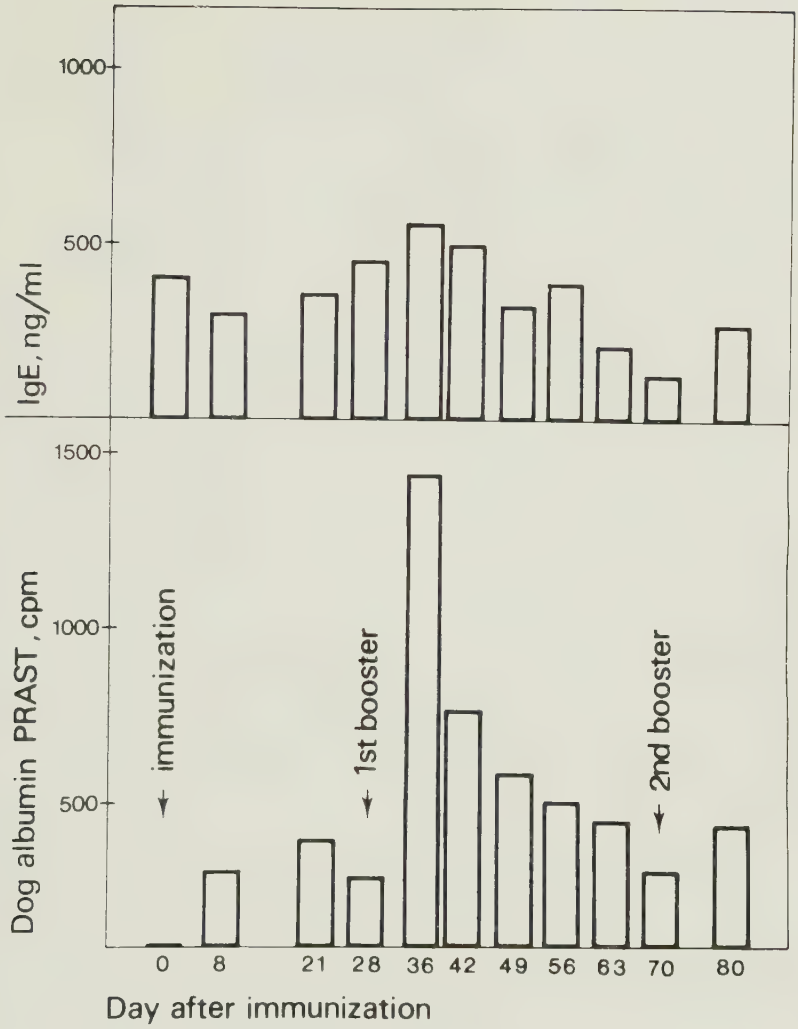


Fig. 27. Total IgE and IgE antibody in serum from 5 Hooded Lister rats immunized with dog albumin in aluminum hydroxide gel. Effect of repeated booster injections of antigen. Ten µg of antigen in 10 mg of adjuvant were given by i.p. injection on Day 1, 29 and 71.

TABLE 16

Variation in the IgE antibody titer in two reference sera, as determined by passive cutaneous anaphylaxis (PCA) in rats of 3 different strains. The sera, obtained from Hooded Lister rats and containing IgE antibody to, respectively, ovalbumin (OA-antigen; 3.727 PRAST-units/ml) and N. brasiliensis (N.b.-antigen; 2.068 PRAST-units/ml) were tested on 5 animals from each strain.

Recipient strain	Age months	Reference serum 1 OA-antigen PCA-titer		Reference serum 3 N.b.-antigen PCA-titer		Total IgE[a] ng/ml
		Mean	Variation	Mean	Variation	
HL	3	512	512-1024	128	128-256	2471
HL	6	2048	2048	512	256-1024	2030
LOU/C	3	512	512-1024	512	256-1024	343
LOU/C	6	1024	512-1024	256	64-512	243
SD	8	1024	1024-2048	128	128-256	2
PCA titer		1024	512-2048	256	64-1024	-

[a] as determined by PRIST

young animals of the same strains, a similar trend was found to be less obvious with respect to the PCA titers obtained for IgE anti-parasite antigen. In the present series of experiments no correlation was observed between the level of total serum IgE and the PCA titer. Although the fact that high PCA titers were obtained in animals having very high total IgE would suggest that there is a sufficient number of free IgE receptors on their mast cells to promote a reaction, this concept, if correct, will not explain why the same, or even higher, PCA titers were not obtained in animals having a low level of serum IgE. Comparison of the PCA titer relative to IgE antibody units, as determined by standardized PRAST, indicates that for ovalbumin one PCA titer unit corresponds to about 4 PRAST units of IgE, whereas for N.brasiliensis antigen one PCA unit is equivalent to 8 PRAST IgE units per ml. When used under standardized conditions, the results obtained by PCA and by PRAST are usually found to be in good agreement, as is illustrated in Table 14 and in Fig. 26. However, since PRAST shows less variation and is more sensitive than PCA, samples that are negative in PCA but positive in PRAST are often found, particularly during the early phase of an immune response. The opposite situation, having a positive PCA and a negative PRAST, has so far not been observed.

5. IgE and the mast cell.

The mediator function of IgE antibody in allergic reactions of Type I (Ishizaka et al., 1967; Johansson and Bennich, 1967) is explained by its unique ability to react both with antigen and with receptor sites on the membrane of blood basophils and tissue mast cells (Ishizaka et al., 1970c; Kulczycki et al., 1974). Several attempts to characterize the putative IgE receptor of human basophils, rat mast cells and, more recently, rat basophilic leukemia cells have been made. Evidence for the attachment of IgE molecules to the membrane of mast(basophil) cells includes release of histamine by antibody specific to IgE (Ishizaka et al., 1971), removal of reaginic activity in serum by absorption with intact mast cells or mast cell extracts (Bach et al., 1971; Bach and Brashler, 1976), external radiolabelling of mast cell membrane proteins and analysis of IgE specific immunoprecipitates by polyacrylamide gel electrophoresis (Conrad and Froese, 1976) and electron microscopy (Sullivan et al., 1971). Attempts to determine the number of receptors for IgE per cell indicate that the number varies between 3×10^4 - 1×10^5 for human basophils (Ishizaka, 1973) and 3×10^5 - 1×10^6 for rat mast cells (Metzger, 1976). Studies of the IgE receptor present on rat basophilic leukemia (RBL) cells indicate that it is likely to represent a glycoprotein (Kulczucki et al., 1976) with a molecular weight in the range of 50,000-77,000 (Conrad et al., 1976; Newman et al., 1977). In the presence of non-ionic detergent (Nonidet P40), which was proven efficient for the solubilization of the receptor in active form (Conrad and Froese, 1976), the molecular weight of the receptor and the receptor-IgE complex has been estimated to 130,000 and 330,000, respectively (Newman et al., 1977). The binding of IgE to the receptor has been found to be reversible and the average equilibrium constant for the reaction has been estimated to be in the order of 10^9-10^{10} M^{-1} at 37°C (Metzger, 1976). The experiments to be described in this section were initiated by the observation of a dissimilarity in the in vitro reactivity

of mast cells isolated from Hooded Lister and Sprague Dawley rats, which justified a comparative study of these cells with respect to uptake of IgE, distribution of membrane protein, content of histamine and induction of release of histamine by various agents.

Uptake of IgE and anti-IgE antibody: For determination of the uptake by mast cells of radiolabelled IgE or anti-IgE antibody, the following protocol was used. Purified mast cells (III:6) isolated from nonimmunized Hooded Lister or Sprague Dawley rats were resuspended in buffer containing the agent under study and incubated at 37°C. After an appropriate incubation period, the cells were carefully washed with buffer, pelleted by centrifugation, resuspended in buffer containing ^{125}I-labelled reagent and finally incubated at 37°C. For removal of unbound reagent, 100 µl of the cell suspension was layered over 100 µl of 29 per cent (w/w) of BSA in PBS in a 0.25 ml polypropylene tube. After centrifugation for 1 min in a microfuge (Beckman Instruments, Inc.), the tube was frozen at -30°C and the tip containing the cell pellet was snipped off and counted. The uptake of protein was calculated from the difference in radioactivity present in the pellet and the supernatant.

In the experiment presented in Table 17, 0.85 x 10^6 mast cells per sample in 900 µl PBS were incubated together with 200 ng of ^{125}I-IgE(IR2) or 150 ng of ^{125}I-anti-IgE antibody in the presence or absence of a 300-fold molar excess of IgE(IR2) or anti-IgE antibody. After 7, 15, 30, 60 and 120 min., 100 µl of each sample were taken and the uptake of radiolabelled protein was measured as described above. As shown in Table 17 about twice as many IgE-binding sites are free on Sprague Dawley cells as compared with Hooded Lister mast cells, which are initially estimated to contain 12-40 times more membrane bound IgE. In the presence of anti-IgE antibody the uptake of IgE on the Hooded Lister cells was very rapid, which might be explained by the formation of complexes between cell-bound and free IgE and antibody. Accordingly, the effect of an excess of anti-IgE antibody on the uptake of IgE on Sprague Dawley mast cells was

TABLE 17

Relative uptake of radiolabelled (^{125}I) rat IgE (A) and rabbit anti-rat-IgE (B) by purified Hooded Lister and Sprague Dawley rat mast cells. Competitive inhibition of the uptake of radiolabelled protein was performed either by addition of an excess amount of unlabelled rat-IgE or rabbit-anti-IgE. The reactions were performed in 10 per cent normal SD serum at 37°C.

	Mast cells	Competitive inhibitor	Reaction time, minutes 7	15	30	60	120
A.	HL	-	0.07	0.16	0.21	0.34	0.59
	HL	rat-IgE (IR2)	0.02	0.02	0.02	0.02	0.03
	HL	rabbit-anti-IgE (IR162)	0.40	0.55	0.64	0.65	0.74
	SD	-	0.13	0.22	0.43	0.64	1.00
	SD	rat-IgE (IR2)	0.05	0.03	0.02	0.02	-
	SD	rabbit-anti-IgE (IR162)	0.04	0.05	0.06	0.06	0.45
B.	HL	-	0.49	0.63	0.80	0.84	1.00
	HL	rat-IgE (IR2)	0.02	0.03	0.04	0.02	0.07
	HL	rabbit-anti-IgE (IR162)	0.03	0.03	0.03	0.03	0.05
	SD	-	0	0	0.02	0.06	0.08
	SD	rat-IgE (IR2)	0.02	0.01	0.03	0.06	0.05
	SD	rabbit-anti-IgE (IR162)	0.02	0.02	0.02	0.06	0.02

different, as there are initially very few IgE molecules bound to these cells. The late onset of IgE binding presumably indicates an inhibition of the receptor-binding function of the IgE molecule by bound antibody. That this inhibition was overcome in time by the complexing effect indicates that the receptor-binding portions of the IgE molecule still were free but now had a different affinity for the receptor.

The uptake of anti-IgE and anti-Fc-ε antibody: Antibody directed against the Fc portion of the IgE molecule was introduced as a probe for the study of the binding of IgE to its mast cell receptor. To compare the uptake of anti-Fc-ε and anti-IgE antibody for mast cell-bound IgE, the following experiment was performed. 4×10^5 Hooded Lister or Sprague Dawley mast cells per sample in 350 µl PBS were incubated with 375 ng of ^{125}I-radiolabelled anti-IgE or 200 ng of ^{125}I-radiolabelled anti-Fc-ε antibody in the presence or absence of a 100-fold molar excess of unlabelled anti-Fc-ε antibody at 37°C for 60 min. As shown in Table 18, the results demonstrate that antibody to Fc-ε reacts with membrane bound IgE with a quantitative uptake approximately 20 per cent greater than that of anti-IgE antibody. However, since anti-IgE constitutes a mixture of antibodies having different specificities as illustrated by the result of the inhibition experiments, the observed difference in uptake may well reflect the result of steric hindrance or the exclusion of antibodies directed against adjacent determinant groups.

Competition between antibody to IgE, $F(ab')_2$-ε and Fc-ε in binding to mast cell bound IgE: Mast cells isolated from Hooded Lister rats were allowed to react with antibody directed against different portions of the IgE molecule: Anti-IgE (a mixture of antibody directed against the whole IgE molecule excluding the light chains), anti-$F(ab')_2$-ε and anti-Fc-ε. 7.5×10^5 mast cells per sample in 800 µl PBS were allowed to react with 15 ng of ^{125}I-labelled antibody of each of the three types in the presence or absence of a 2,000-fold molar excess of unlabelled antibody of each type at 37°C. After 30, 60, 120 and 270 min,

TABLE 18

An examination of the uptake of radiolabelled (^{125}I) rabbit-anti-rat IgE and rabbit anti-Fc-ε by Hooded Lister and Sprague Dawley mast cells and the competitive effect of adding an excess amount of unlabelled rabbit anti-rat-Fc-ε. All incubations were performed in 10 per cent SD serum for 60 min. at 37°C.

Antibody	HL mast cells ng Ab/10^6	% inhib.	SD mast cells ng Ab/10^6	% inhib.
(^{125}I)anti-IgE	16.70	-	1.58	-
" + anti-Fc-ε	9.38	43.8	0.89	43.7
(^{125}I)anti-Fc-ε	25.55	-	1.79	-
" + anti-Fc-ε	2.70	89.4	0.11	93.9

100 µl of sample were taken for determination of the uptake of radiolabelled antibody. As illustrated in Table 19 and in Fig. 28, the most interesting result was that anti-F(ab')$_2$-ε inhibited the binding of anti-Fc-ε to a greater extent than anti-Fc-ε inhibited the binding of anti-F(ab')$_2$-ε, the relative inhibition being 76 and 34 per cent, respectively, which indicates that some of the binding sites for anti-Fc-ε antibody may become less accessible when membrane bound IgE interacts with anti-F(ab')$_2$ antibody.

TABLE 19

Competition between rabbit antibody, reactive to different regions of the IgE molecule, for binding to mast cell bound IgE. The reaction was performed in 10 per cent normal SD serum pH 7.2 at 37°C using Hooded Lister mast cells.

^{125}I antibody	Inhibitor	Relative uptake Time, minutes			
		30	60	120	270
anti IgE	-	0.45	0.71	0.83	1.00
	anti IgE	0.08	0.06	0.04	0.03
	anti $F(ab')_2$-ε	0.30	0.18	0.16	0.21
	anti Fc-ε	0.24	0.18	0.18	0.20
anti $F(ab')_2$-ε	-	0.61	0.80	0.95	1.00
	anti IgE	0.13	0.09	0.06	0.06
	anti $F(ab')_2$-ε	0.14	0.10	0.08	0.08
	anti Fc-ε	0.53	0.52	0.51	0.66
anti Fc-ε	-	0.46	0.61	0.69	1.00
	anti IgE	0.05	0.05	0.03	0.03
	anti $F(ab')_2$-ε	0.28	0.23	0.23	0.24
	anti Fc-ε	0.08	0.06	0.05	0.08

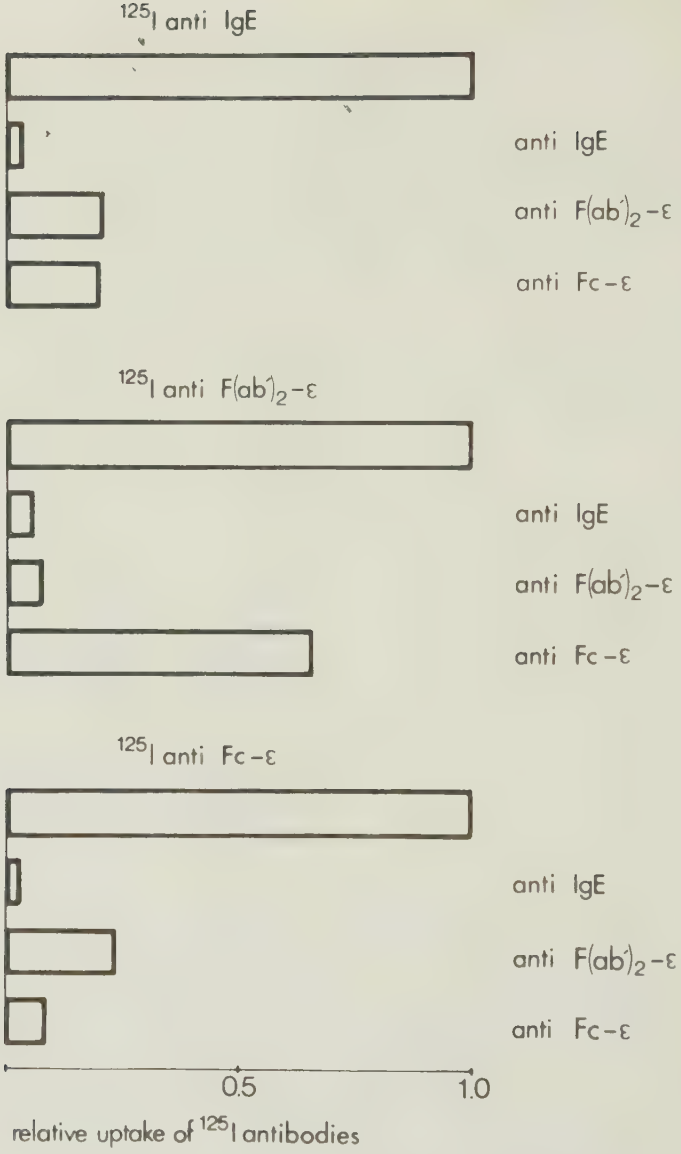


Fig. 28. Competition of rabbit antibody specific for different antigenic determinant groups on rat IgE for binding to mast cell bound IgE. For experimental conditions, see Table 19 .

Uptake of IgE and anti-Fc-ε by acid treated mast cells: Purified mast cells from Hooded Lister rats were incubated in isotonic buffer, pH 3 at 20°C, as described by Ishizaka and Ishizaka (1974). After an incubation period of 60 sec the cells were washed in several ml of PBS. An aliquot of the acid treated cells and a control sample of untreated cells were incubated in 500 µl of 10 per cent Sprague Dawley serum for 30 min at 37°C, while an additional aliquot of treated cells were incubated with 100 µg of IgE(IR2). The cells were then washed in PBS and the samples, containing 6 x 10^5 cells per sample, were incubated at 37°C with 100 ng of ^{125}I-labelled IgE(IR2) or 150 ng of ^{125}I-labelled anti-Fc-ε in 500 µl of 10 per cent Sprague Dawley serum. After 30, 60, 120 and 180 min, a 100 µl aliquot from each sample was taken for analysis of the uptake of the radiolabelled reagent. As shown in Table 20, about 65 per cent of membrane bound IgE was removed by treating the cells at pH 3 but the brief exposure to acid conditions did not appear to impair the mast cell receptor function as 3 times as much IgE was bound after acid treatment than before. However, the results obtained by using anti-Fc-ε antibody seem to be in conflict with the presumption that most IgE receptors should be saturated after incubation of the cells with an excess amount of IgE. One explanation for only about 60 per cent of the expected amount of IgE being bound is that loosely associated protein still remaining after washing reacts with antibody and the complex is then left in the supernatant when the cells are freed from excess reagent. Another explanation might be that a portion of the IgE receptors may become unreactive as a result of the acid treatment.

Uptake by mast cells of heat-treated IgE: Reaginic antibody loses homocytotropic activity when heated at 56°C for 30-60 min (Ishizaka et al., 1967) due to an irreversible conformational transition in the Fc portion of the molecule (Bennich and Dorrington, 1972). To determine the uptake by mast cells of heat-treated rat myeloma IgE, the following experiment was undertaken. 5 x 10^5 purified Hooded Lister mast cells were incubated for 150 min at 37°C with 50 ng of normal or heat-treated (56°C, 30 min)

TABLE 20

Relative uptake of radiolabelled (^{125}I) rabbit anti-rat IgE (IR162) and anti-rat Fc-ε on Hooded Lister mast cells, before and after treatment of the cells for 60 sec at pH 3.0, 20°C. All incubations were performed in 10 per cent normal SD serum, pH 7.2 at 37°C.

^{125}I reagent	Preincubation in buffer pH	Preincubation	Time, minutes 30	60	120	180
Rat IgE (IR2)	7.2	-	0.08	0.17	0.29	0.3
	3.0	-	0.35	0.59	0.89	1
	3.0	Rat IgE (IR2)	0.01	0.02	0.04	0.0
Rabbit anti-rat Fc-ε	7.2	-	0.50	0.77	0.93	1
	3.0	-	0.12	0.18	0.33	0.3
	3.0	Rat IgE (IR2)	0.31	0.40	0.54	0.5

radiolabelled IgE(IR2) in 500 µl of 10 per cent Sprague Dawley serum. The results presented in Table 21 show that essentially no uptake of heat-treated IgE was obtained by using either normal or acid-treated mast cells.

The effect of serum factors on the uptake of IgE by mast cells:
To determine if the presence of serum factors might have an influence on the uptake of IgE by mast cells. Purified mast cells from Hooded Lister and Sprague Dawley rats were incubated in autologous serum and the uptake of IgE was compared with that obtained when the cells were incubated in PBS. 10^6 mast cells in 100 µl PBS were added to 500 µl serum or PBS containing 25 ng of radio-

TABLE 21

Relative uptake of heat-denatured and native rat ^{125}I-IgE (IR2) by Hooded Lister rat mast cells, before and after preincubation of the cells in pH 3.0 buffer for 60 sec at 20°C. The reactions were performed in 10 per cent normal SD serum, pH 7.2 at 37°C.

^{125}I-IgE (IR2)	Mast cells preincubation	Time, minutes 30	60	150
Native	pH 7.2	0.06	0.15	0.34
	pH 3.0	0.24	0.54	1
Heated to 56°C, 30 min	pH 7.2	0.01	0.01	0.02
	pH 3.0	0.01	0.01	0.06

labelled IgE(IR2) and the samples were then incubated for 150 min at 37°C. The relative uptake of IgE by the samples is listed in Table 22. These results demonstrate that the presence of serum had a suppressive effect on the uptake of IgE by both Hooded Lister and Sprague Dawley mast cells with the uptake of IgE only about one half of that obtained in PBS. The difference in uptake obtained in the presence of serum is explained by a difference in total IgE, being 180 ng per ml in Hooded Lister and 0 in Sprague Dawley. More surprising was the finding of a comparatively small difference in uptake between Hooded Lister and Sprague Dawley cells when incubated in PBS.

TABLE 22

Effect on the uptake of ^{125}I-labelled rat IgE (IR2) by Hooded Lister and Sprague Dawley rat mast cells by incubation in PBS and autologous sera from Hooded Lister and Sprague Dawley rats, respectively.

Incubation medium	Mast cells	Relative uptake
PBS	SD	1
PBS	HL	0.88
SD serum	SD	0.56
SD serum	HL	0.36
HL serum	SD	0.52
HL serum	HL	0.31

Membrane proteins: It has been suggested that IgE receptor molecules present on the mast cell membrane are proteins and therefore it should be possible to label them with ^{125}Iodine by the lactoperoxidase method, provided that the necessary amino acids are exposed (Conrad and Froese, 1976). Differences between surface labelled IgE loaded and IgE depleted mast cells should be detectable and could be used in the study of receptor molecules. To survey the membrane proteins on rat lymphoid cells, different cell types from Hooded Lister rats were compared after ^{125}I-labelling and SDS-gel filtration. The results, summarized in Fig. 29, demonstrated that purified mast cells and mast cell depleted peritoneal cells generally resembled each other but had labelling patterns that were quite different from other cell types. The elution patterns obtained with spleen, lymph node and thymus cells were alike with respect to the positions of major peaks but the amount of radioactivity in the peaks differed. Red blood cells were quite different from all the other cell types examined.

For solubilization of the IgE-receptor membrane complex, 0.5 per cent NP40 in PBS has been used (Conrad and Froese, 1976; Kulczycki et al., 1976). Since mast cells from Hooded Lister and Sprague Dawley rats differ in the amount of membrane bound IgE, as demonstrated previously, a comparative analysis of NP40 extracted radiolabelled cells was done by SDS-gel filtration (III:19B). As illustrated in Fig. 30, the major difference observed was that the supernatant fraction from Hooded Lister cells contained more radioactive material in the first peak, corresponding to the position of native IgE, than the supernatant from Sprague Dawley mast cells. The prominent peak at Ve = 0.53Vt seen with the pellet fractions of both cell extracts contained a group of components with mol.w. about 30,000 which appear to be characteristic for the mast cell.

The effect of acid treatment of Hooded Lister mast cells before radiolabelling is illustrated in Fig. 31. The prominent peak of IgE obtained with the untreated control cells was virtually

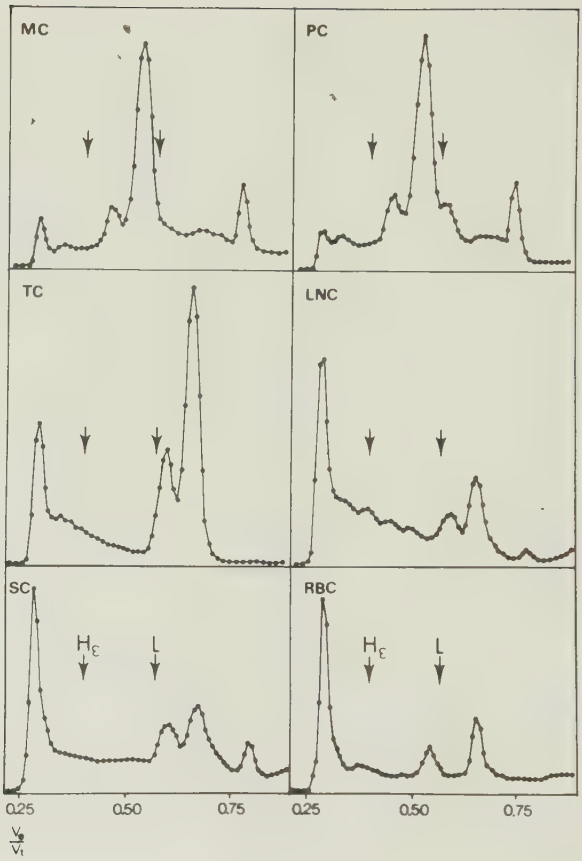


Fig. 29. SDS-Gel filtration analysis of ^{125}I-labelled membrane components from various cell types of the Hooded Lister rat. After radiolabelling, using the lactoperoxidase method, cells (10^7/0.2 ml) were treated with 3 per cent SDS for 10 min at 100°C, centrifuged (20,000 x g) and the supernatant (diluted to 1 ml) applied on a 14 x 900 cm column of Ultrogel AcA 44 in 0.2 per cent SDS-Fairbanks buffer, pH 7.4. 1 ml fractions were collected at a flow rate of 6 ml per hr. MC = peritoneal mast cells; PC = peritoneal cells minus mast cells; TC = thymus cells; SC = spleen cells after removal of red blood cells; RBC = red blood cells. Arrows indicate the positions of heavy (Hε) and light (L) polypeptide chains of monoclonal rat IgE(IR2).

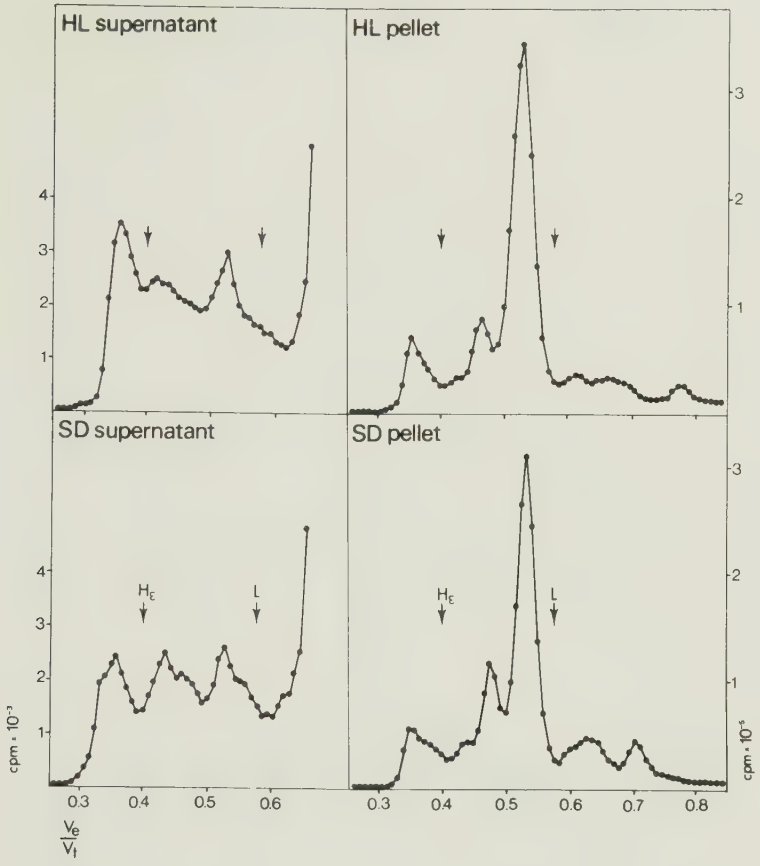


Fig. 30. SDS-Gel filtration analysis of ^{125}I-labelled membrane components from Hooded Lister and Sprague Dawley rat mast cells. After radiolabelling, using the lacto-peroxidase method, the cells were treated with NP-40 and the extract centrifuged at 20,000 x g, 10 mins. Both the pellet and the supernatant were treated with SDS and applied on Ultrogel AcA-44 as described in the legend to Fig. 30.

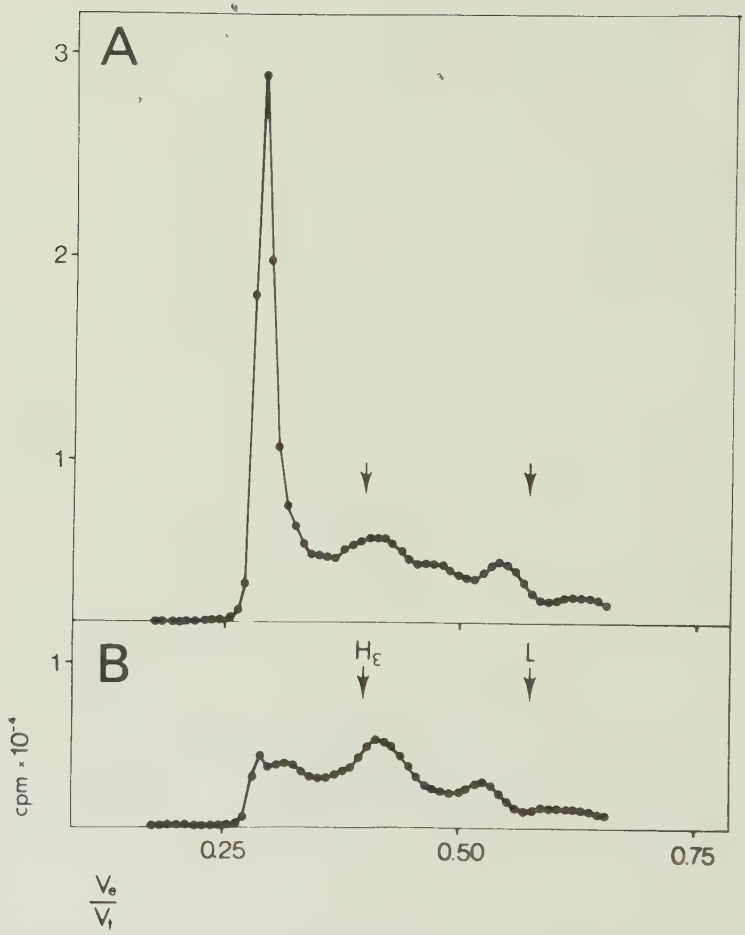


Fig. 31. SDS-Gel filtration analysis of ^{125}I-labelled Hooded Lister rat mast cells saturated with monoclonal IgE(IR2). Effect of pH 3 treatment before radiolabelling.

A. Supernatant of NP-40 extracted cells not subjected to pH 3 treatment before radiolabelling; (Control).

B. Supernatant of NP-40 extracted cells incubated at pH 3 for 60 sec at RT before radiolabelling.

For experimental details see legend to Fig. 30.

absent in the extract of acid treated cells. The experiment shown in Fig. 32 was performed to determine the distribution of radiolabelled components that could be removed by brief exposure of the cells to pH 3 buffer. The results, which should be compared with those obtained by SDS-polyacrylamide gel electrophoresis and autoradiography (Fig. 33) demonstrated that besides the one major component having the chromatographic and electrophoretic characteristics of IgE, very little radiolabelled material was found to be removed by acid treatment. Fig. 34 illustrates the distribution of radiolabelled material present in the NP40 soluble fraction of mast cells when the cells had been labelled with ^{125}I either before or after exposure to acid pH. Although no straightforward explanation can be given for the observed differences, the elution pattern obtained after acid treatment might be the result of exposure of previously masked reactive sites to the radiolabel as well as possibly reflecting the dissociation or breakdown of integral membrane components. That no major differences have occurred in membrane structure or function, as a result of acid treatment is suggested by the observation that mast cells remain viable after pH 3 treatment.

Conrad et al. (1976) have determined the molecular size of the IgE receptor on rat mast cells by gel filtration of the NP40-solubilized IgE-receptor membrane complex. Although the procedure is based on a straightforward concept, it was found difficult to reach unambiguous conclusions using this technique. The results shown in Fig. 35 demonstrated that virtually all surface radiolabelled membrane components solubilized by the NP40 treatment of either mast cells or red blood cells from Hooded Lister rats elute in one major peak at a position corresponding to a molecular size of 3-5 x 10^5 daltons. The NP40 extracts of radiolabelled mast cells also contained material eluting at the void volume (Fig. 35B). The two mast cell peaks were further examined by SDS-gel filtration analysis as shown in Fig. 36. The results demonstrated that the elution profiles of peak 1 and 2 after treatment with SDS were very similar to the elution patterns obtained by SDS-gel filtration analysis of the pellet and the supernatant fractions of NP40 solubilized mast cells.

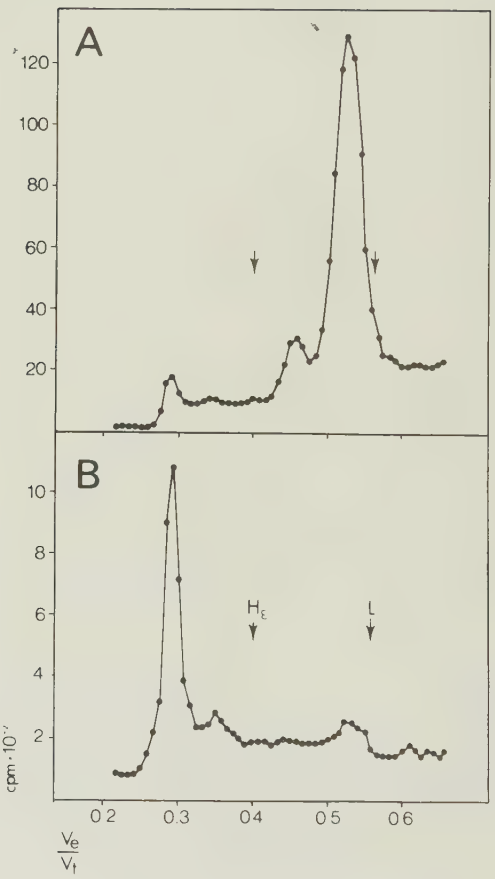


Fig. 32. SDS-Gel filtration analysis of ^{125}I-labelled Hooded Lister rat mast cells. Effect of pH 3 treatment after external radiolabelling.

A. Pellet isolated by centrifugation after incubation of the cells at pH 3 for 60 sec at RT.

B. Supernatant after incubation at pH 3 as described for A, above.

Experimental conditions as described in legend to Fig. 30.

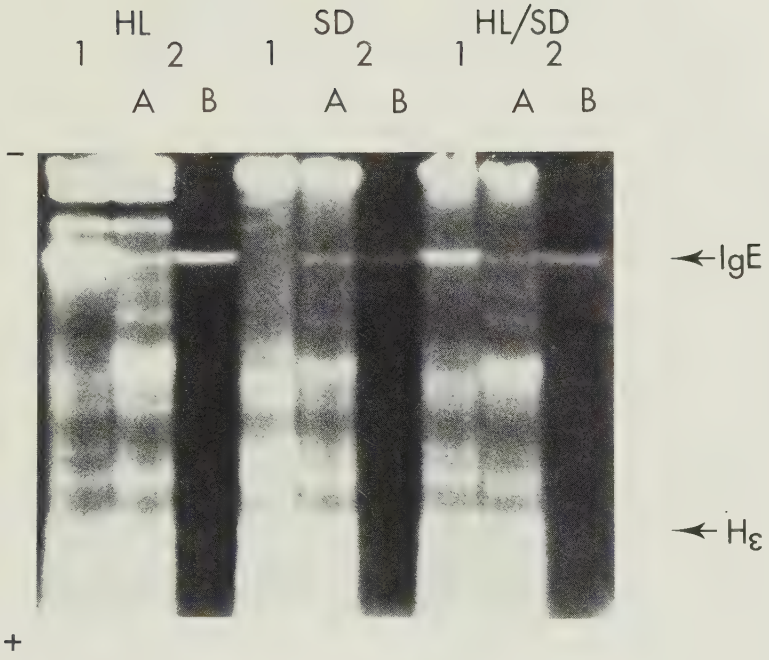


Fig. 33. Membrane bound IgE on mast cells from Hooded Lister, Sprague Dawley and HL/SD F1 hybrid rats, as determined by external radiolabelling with ^{125}Iodine, SDS-PAGE and autoradiography.

1: Pellet of ^{125}I-labelled cells.

2: Mast cells saturated with monoclonal IgE(IR2) before radiolabelling; ^{125}I-labelled cells were treated by exposure to pH 3 buffer for 60 sec at RT, followed by centrifugation.

2A: Cell pellet; 2B: Supernatant.

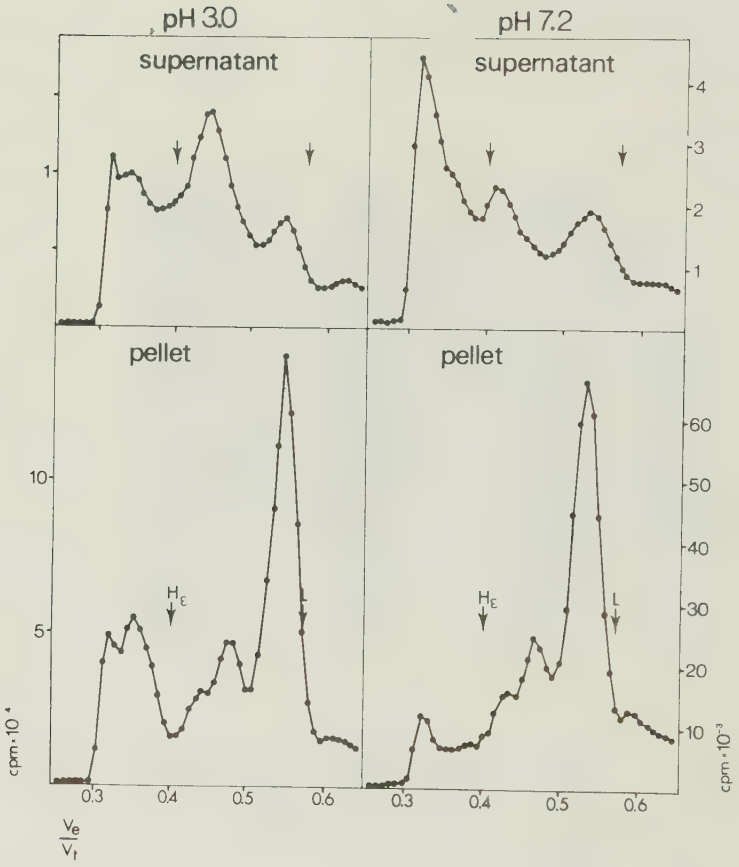


Fig. 34. SDS-Gel filtration analysis of Hooded Lister mast cell membrane components radiolabelled with ^{125}Iodine before and after brief exposure to pH 3 buffer. Cells were extracted with NP-40, centrifuged at 20000 x g, 10 min, and the pellet and supernatant treated with SDS as described in Fig. 30.

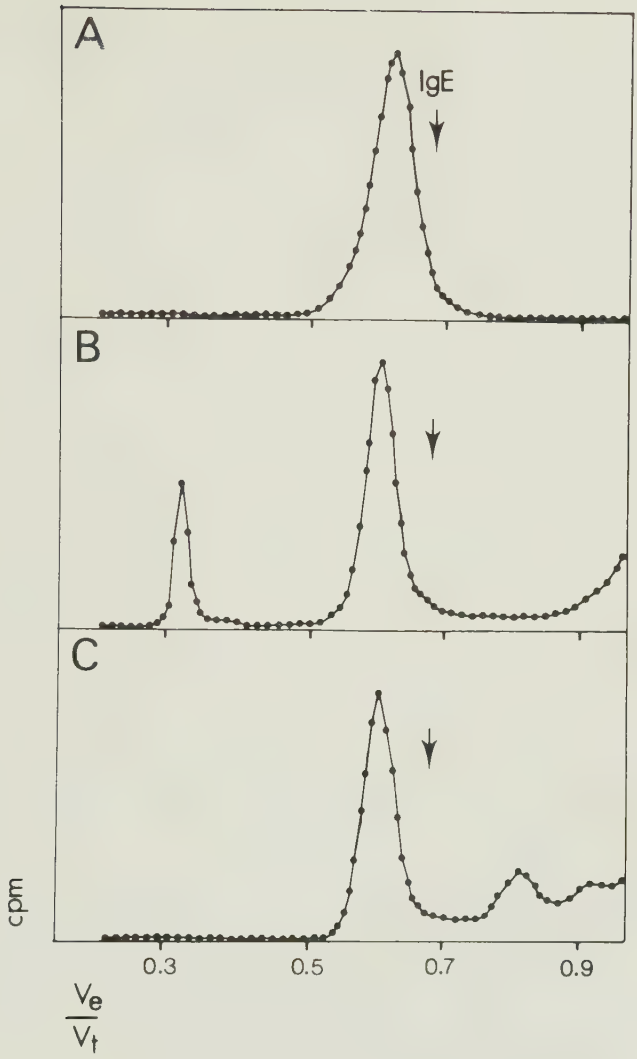


Fig. 35. Gel filtration on Sepharose 6B in 0.5 per cent NP-40,0.1 M Tris-HCl, pH 8 of NP-40 extracted membrane components from A: Hooded Lister rat mast cells incubated with ^{125}I-labelled monoclonal rat IgE(IR2); B: ^{125}I-labelled Hooded Lister mast cells; C: ^{125}I-labelled Hooded Lister red blood cells. The arrow indicates the elution volume for ^{125}I-labelled monoclonal IgE(IR2) subjected to incubation with Hooded Lister red blood cells, and extracted with NP-40.

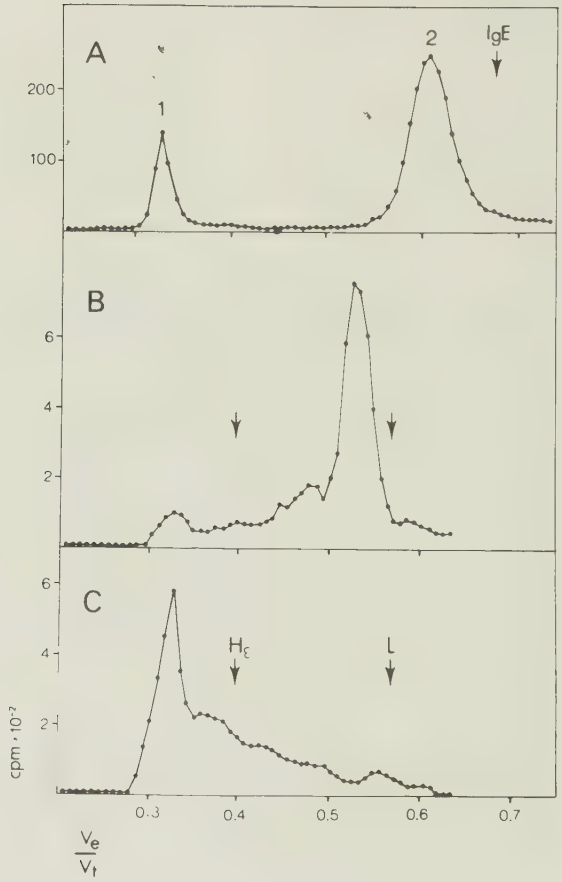


Fig. 36. SDS-Gel filtration analysis of NP-40 extracted mast cell membrane components isolated by separation on Sepharose 6B in 0.5 per cent NP-40 - Tris buffer, pH 8.

A: ^{125}I-labelled Hooded Lister mast cells extracted with NP-40 and fractionated on Sepharose 6B.

B: Fraction 1 from experiment A, above, subjected to SDS-gel filtration analysis on Ultrogel AcA 44 as described in the legend to Fig. 30.

C: Fraction 2 from experiment A, above subjected to SDS-gel filtration analysis as described in B.

An alternative approach to study the mast cell membrane receptor for IgE has been to use classic double antibody techniques. Such procedures combined with SDS-PAGE has successfully been applied by Conrad and Froese (1976) to characterize the IgE receptors from normal rat mast cells and from rat basophilic leukemia (RBL) cells. Albeit very simple in theory, the practical results of immunoprecipitation of membrane components have not always been in concert with the expected outcome, as illustrated in Fig. 37. By immunoprecipitation of radiolabelled Hooded Lister mast cells, three radioactive peaks were resolved by SDS-gel filtration analysis. Although three radioactive peaks were also observed in the experiments of Conrad and Froese, the quantitative distribution of radioactivity was quite different. Thus, while they found a major radioactive peak corresponding to a molecular size of 62,000 daltons, the major radioactive peak obtained in the experiment shown in Fig. 37A eluted at a position corresponding to a molecular weight of 25,000-30,000 daltons. However, the latter peak cannot be related to the IgE receptor as it was also present as the major radioactive constituent in non-IgE specific immunoprecipitates (Fig. 37B). Conrad and Froese, who observed a similar low moleculear weight component in their rat mast cell experiments, speculate that it may represent "some type of 'hetero' Fc recptor" for rabbit or goat immunoglobulin. However, this seems unlikely on quantitative grounds, as the results of the present study demonstrate that it represents a major fraction of surface labelled mast cell membrane components (Fig. 29, 30, 36). The observation that the low molecular weight component is not found in RBL cells (Conrad and Froese, 1976) is particularly interesting in view of the fact that these cells are defective with respect to histamine release (Metzger, 1976), and further studies on the potential function of this unique mast cell protein, might therefore be rewarding. The fact that the results in Fig. 37A demonstrated only a small peak of radioactivity at the position expected for a putative IgE receptor molecule of molecular weight 62,000, (i.e. Ve = 0.42Vt) might reflect that a considerable fraction of the receptor sites were initially occupied by in vivo IgE. The finding of a highly radioactive peak in the position of IgE, obtained only by specific immunoprecipitation, gives support to this possibility. However, a series of experiments with Sprague Dawley cells have also resulted in a low recovery of radiolabelled receptor molecules, which indicates that the explanation must be sought elsewhere.

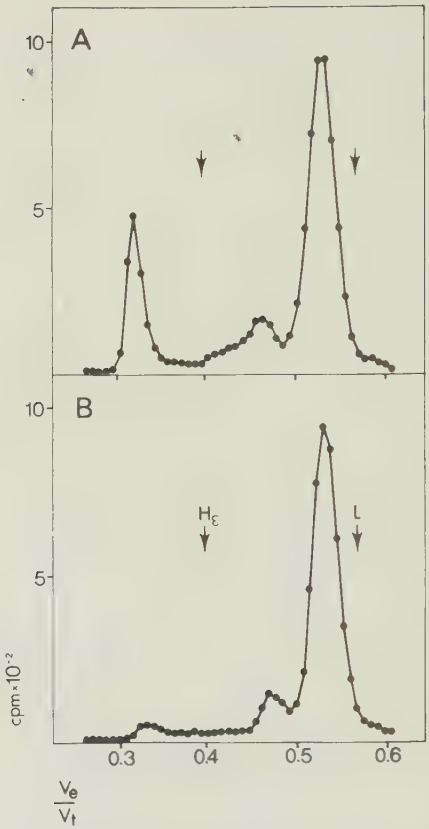


Fig. 37. SDS-Gel-filtration analysis of membrane components, isolated by immunoprecipitation, from Hooded Lister rat mast cells extracted with NP-40 after external radiolabelling with ^{125}Iodine.

A: To an NP-40 extract (supernatant) of ^{125}I-labelled Hooded Lister mast cells, mixed with unlabelled monoclonal rat IgE(IR2), was added a known amount of immunosorbent purified rabbit anti-rat IgE(IR162) followed by an equivalent amount of goat anti-rabbit IgG. After incubation for 30 min at 37°C the immune complex was isolated by centrifugation and treated with SDS for 2 min at 100°C and analysed on Ultrogel AcA 44 as described in Fig. 30.

B: The experimental conditions were as described in experiment A above, except that specific rabbit antibody to rat IgE was replaced by purified rabbit IgG isolated from nonimmunized animals.

Histamine release: Many research groups studying the histamine release phenomenon have focused their interest on the isolated rat peritoneal mast cell. Mast cells can readily be prepared in 95-100% purity from peritoneal and pleural washings, containing 5-10% mast cells, by density gradient centrifugation on Ficoll or BSA (III:6). Mast cells in vitro can be induced to release histamine by interaction with various agents. Anti-IgE can be used to mimic the situation where allergen couples to IgE antibody molecules on the mast cell membrane. Mast cells can be passively sensitized by IgE antibody and then challenged with the appropriate allergen. The polyamine, Compound 48/80, will release histamine from mast cells independent of the IgE-allergen reactions. Concanavalin A (ConA) has been reported to release histamine from mast cells (Keller, 1973; Sullivan et al., 1975a) but the reactions involved are not known in detail. Phosphatidyl serine (PS) is known to be an enhancer of histamine release (Goth et al., 1971).

To investigate if the differences between Hooded Lister and Sprague Dawley rats could be extended to mast cell reactivity, experiments were performed using the histamine releasing agents mentioned above (Tables 23 and 24). Mast cells were isolated both by the BSA and Ficoll fractionation methods and the purified cells tested to determine if the isolation technique affects the outcome of histamine release experiments. Hooded Lister mast cells isolated by the methods mentioned were incubated with Compound 48/80 (1 µg per ml) or with dilutions of anti-IgE antibodies. As shown in Table 23, although the immune reactivity was higher when BSA fractionated cells were used, the spontaneous histamine release was also greater. Compound 48/80 released more histamine from BSA cells than from Ficoll cells.

The immune reactivity of BSA fractionated Hooded Lister (HL) and Sprague Dawley (SD) mast cells was investigated with the following experiment (see III:17). The cells were divided in two groups

TABLE 23

Difference in histamine release by Hooded Lister rat mast cells isolated by gradient centrifugation in, respectively, bovine serum albumin (BSA) and Ficoll.

		BSA	FICOLL
Histamine release in % of total. (Background subtracted)			
48/80	1 µg/ml	56.3	49.4
anti-IgE	10^{-1} mg/ml	5.8	5.2
	10^{-2}	19.6	10.2
	10^{-3}	16.9	5.3
	10^{-4}	5.1	3.7
Background release		0	0
Histamine left in cells after preparation (% of histamine in mast cells before prep.)		97.8	97.0

TABLE 24

Effect of phosphatidylserine (PS) on the release of histamine from normal (A) and passively sensitized (B) Hooded Lister and Sprague Dawley rat mast cells, challenged with rabbit anti-rat IgE, concanavalin A (ConA) and antigen (Ovalbumin).

		A.				B.			
Reagents	Conc. mg/ml	% Histamine release[x] HL		SD		% Histamine release[x] HL		SD	
		normal	+PS	normal	+PS	normal	+PS	normal	+PS
Anti IgE	10^{-1}	26.3	46.3	0	1.5	33.4	53.2	4.1	10.1
	10^{-2}	24.7	41.3	0	0.9	24.8	43.7	2.8	6.3
	10^{-3}	15.6	28.3	0	0.6	8.1	35.1	0	0
	10^{-4}	0	8.5	0	0	0	11.6	0	0
Con A	10^{-1}	20.6	37.4	0	7.3	21.0	27.8	0.5	1.7
	10^{-2}	7.4	23.2	0	3.6	8.2	28.6	0	0
	10^{-3}	0	3.9	0	2.3	0	9.3	0	0
	10^{-4}	2.2	3.4	0	2.1	1.9	5.6	0	0
Ovalbumin	5×10^{-1}	0	0	0	0	0	1.3	0	0
	5×10^{-2}	0	0	0	0	2.2	5.9	0	0
	5×10^{-3}	0	0	0	0	3.2	13.5	0	0
	5×10^{-4}	0	0	0	0	3.3	16.6	0	0.1
Background		3.0	2.6	2.5	1.6	0.4	3.2	2.7	4.3

[x] background release subtracted

TABLE 25

Effect of phosphatidylserine (PS)on the release of histamine from passively sensitized Hooded Lister and Sprague Dawley rat mast cells challenged with 1 µg/ml of Compound 48/80.

Reagent added	Histamine release, %	
	HL	SD
-	64.1	43.6
PS 175 µg/ml	20.4	0

(A and B) and incubated for 30 minutes at 37°C in MCM-7 buffer containing 17% HL serum (A - a Hooded Lister normal serum, 500 ng IgE per ml, and B - a Hooded Lister immune serum, 1050 OA-PRAST units per ml, 150 µg IgE per ml). After washing, half of each sample was incubated in MCM-7 buffer containing 175 µg per ml of phosphatidyl serine (PS) for 15 minutes at 37°C. All cell samples were then challenged with varying concentrations of anti-IgE, ConA or OA. The results (Table 24) show that the reactivity of HL mast cells after challenge with all threee agents was better than that of SD mast cells. This held for PS treated cells as well. Nonsensitized cells gave no histamine release when reacted with OA. Mast cell reactivity when stimulated with Compound 48/80 was descreased in PS treated cells (Table 25).

Skin tests: As PRAST was used to study IgE antibodies directed against different antigens, only a few experiments with PCA and other skin tests were performed. The relationship between PRAST and PCA values has previously been discussed and only some details about skin reactivity will be mentioned below. It has been reported that IgE tumour bearing LOU rats cannot be used in PCA tests (Bazin and Beckers, 1976) and that human IgE myeloma protein inhibits PK tests (Stanworth et al., 1967). PCA tests can also be inhibited by the addition of increasing amounts of IgE(IR2) myeloma protein which competes with IgE antibody for the free receptor sites on skin mast cells.

The difference in PCA response between 3 Hooded Lister rats with normal IgE levels (320 ng per ml) and 3 LOU rats with overly high IgE levels (7500 ng per ml) was studied. 50 µl of OA positive serum (3727 OA-PRAST units per ml and total IgE, 7 µg per ml) at 1/512 dilution (7.3 PRAST units per ml) were injected i.c. together with 0, 1, 10, 100 or 1,000 µg IgE(IR2) per ml. The rats were challenged with OA after 48 hrs. Only the HL rats showed positive PCA, but only samples at the lowest IgE(IR2) concentration or containing no IgE(IR2) at all showed positive PCA reactions (Table 26). The antibody level per sample was 0.4 PRAST units and the total amount IgE 0.7 ng. A 125 fold molar excess of IgE(IR2) over IgE antibody had no inhibitory effect, whereas a 1250-fold molar excess inhibited the PCA reaction completely. This is analogous to the situation when sera from N. brasiliensis infected rats immunized with OA antigen are tested. In these cases, if the sera contain 10 OA PRAST units per ml and the total level of IgE is 100 µg per ml, the nonantibody IgE is 10,000 fold the IgE antibody, so weak or negative reactions would be expected in PCA.

Direct skin tests were performed to study the difference between HL and SD rats in the levels of IgE bound to mast cell receptors. As shown above in binding studies, although very few SD mast cell IgE receptors are filled, the total number of receptors seem to be

TABLE 26

Competitive inhibition between monoclonal rat IgE and serum IgE antibody, as determined by passive cutaneous anaphylaxis (PCA) in Hooded Lister and LOU/C rats. 50 µl of OA positive serum (7.3 PRAST units per ml) injected per site together with different amounts of myeloma IgE(IR2).

IgE (IR2), ng/50 µl	PCA reactions	
	Hooded Lister[a)]	LOU/C[b)]
0	+	-
50	+	-
500	-	-
5000	-	-

[a)] HL serum IgE = 0.32 µg/ml [b)] LOU/C serum IgE = 7.5 µg/ml

TABLE 27

Reversed type PCA reactions in Hooded Lister and Sprague Dawley rats, after challenge with rabbit antibody of different specificities.

Rabbit antibody against	Skin response	
	HL	SD
Rat IgE (IR162)	+	-
Rat $F(ab')_2$-ε	+	-
Rat Fc-ε	++	-
HL rat mast cells	++	+
Rat IgG (normal)	-	-

the same on SD and HL mast cells. HL and SD rats were injected i.c. with three different types of anti IgE antibodies, one anti IgG and one anti RMC(HL) serum. All sera were titrated over a wide range. 20 - 30 min after the injections, 1 ml of 1% Evans blue dye in PBS was injected i.v. and the result was inspected 5 min later (Table 27). The results indicated that there were very few or any IgE molecules on SD mast cell membrane receptors. The anti RMC used as a control showed that the SD mast cells were reactive.

V. DISCUSSION

The radioimmuno assays for determining total IgE (PRIST) and IgE antibody (PRAST) in rat serum presented in this thesis have made it possible to measure IgE levels below 1 ng per ml. Other assays used for total IgE in rat serum, such as the ammonium sulphate precipitation technique (Carson et al.,1975a) and the radioimmuno single radial diffusion procedure (Rowe, 1969; Jarrett and Bazin, 1974), have mostly been used to follow the increase of total serum IgE after parasitic infection and therefore, a high sensitivity has not been necessary since infection with, for example, Nippostrongylus brasiliensis larvae increases the serum IgE 1,000-fold (up to a level of 100-300 µg per ml). The use of PRIST, as described in this thesis, has demonstrated significant differences in normal serum IgE levels between rat strains. Serum IgE in young Hooded Lister rats is undetectable before day 13 but increases to levels normal for the strain within 2 to 3 months. No differences in serum IgE levels between females and males can be seen. Environmental factors seem to have a pronounced influence on the serum IgE level so that optimal housing conditions give low and relatively stable IgE levels compared to the great fluctuations of IgE levels seen in rats from animal houses with lower standards. Even within a strain, a wide range of IgE levels can be seen even though the rats are kept in the same animal room. Additionally, all rats pass through stages with higher than normal IgE levels now and then during their lifetime. PRIST has also been used to follow the production of IgE by IgE myeloma cell lines in vitro (Bennich et al., 1978).

To determine IgE antibody, the only assay available until recently has been passive cutaneous anaphylaxis (PCA). Small quantitative differences in IgE antibody cannot be discovered by PCA and the outcome often depends more on technical errors than on different IgE antibody levels.

Some of the advantages with PRAST, as compared to PCA, are:

1) the ability to process a large number of samples identically,
2) the possibility, by using a standard serum, to compare results

obtained over an extended period, 3) the ability to correlate the IgE antibody level to fluctuations in total serum IgE (as measured by PRIST) and 4) approximately 200 times less antigen (allergen) is required per sample. Thus, by using PRIST and PRAST it was possible to follow even small variations in IgE. PRAST has also been used to measure the relative levels of IgG antibody after immunization and thus it is possible to compare the IgE and IgG antibody responses within the same test system.

Attempts have been made to measure mouse IgE with rat IgE reagents, however, the low cross-reactivity between rat and mouse IgE in PRIST and PRAST have hampered the determination of the IgE level in mouse serum.

Differences in the IgE immune response between strains within the same species have been previously reported (rat - Murphey et al., 1974; Gasser et al., 1975; mouse - Levine and Vaz, 1970). In the present study a relationship has been found between the basic IgE level and the immune response. That is, a low normal IgE level strain demonstrates a weak IgE response after parasitic infection and immunization and a low mast cell reactivity whereas a high normal IgE level strain reacts strongly in both cases.

PCA titers obtained in high and low IgE level strains showed no differences, indicating that free IgE receptors are present in both cases. Overly high serum concentrations of IgE in high IgE level rats (e.g. LOU), or addition of myeloma IgE to samples, inhibit PCA reactions in the same fashion as an excess of myeloma IgE in IgE tumour bearing rats, has been reported to do (Bazin and Beckers, 1976). Therefore, when high IgE level rats are used for PCA testing it is important to know the infection status (IgE level) of every animal used in order to be confident about the results. To use low IgE level rat strains for PCA tests is obviously safer, as these rats never reach IgE levels high enough to block all the IgE receptors.

With the use of radiolabelled rat IgE myeloma protein and antibodies directed against the IgE molecule, it has been shown that not all IgE receptors on Hooded Lister and Sprague Dawley mast cell membranes are occupied in vivo. In vitro, all the receptors can be occupied by addition of IgE myeloma protein in excess. By PRIST, the amount of IgE dissociated from normal Hooded Lister mast cells by low pH treatment has, for the first time, been quantified. Low pH treatment, first performed on human basophil leukocytes by Ishizaka and Ishizaka (1974), was, when applied to rat basophil leukemia cells (Kulczycki and Metzger, 1974), shown to leave up to 20% of the IgE molecules on the cells. With the same treatment of Hooded Lister mast cells only about 2/3 of the IgE, corresponding to 15,000-20,000 molecules per mast cell (as measured by PRIST) were dissociated. It was not possible to measure the small amount of IgE present on Sprague Dawley mast cells. The total number of IgE receptors per mast cell has not been determined but calculation based on IgE and anti-IgE uptake and the measurements of low pH dissociated IgE gives a figure between 25,000 and 75,000 IgE receptors per mast cell. These experiments have demonstrated no difference between Hooded Lister and Sprague Dawley rats with respect to the number of IgE receptors per mast cell. The difference in the amount of IgE on the mast cell membrane in vivo corresponds directly to the differences in serum IgE levels.

It has been suggested that the Fc region of the IgE molecule participates in the binding to IgE receptors (Stanworth et al., 1968; Ishizaka et al., 1970a). Experiments with antibodies against different regions of the rat IgE molecule show that the Fc region of receptor bound IgE is less accessable for reaction with antibody than the $F(ab')_2$ region.

Surface radio-labelling and SDS gel filtration of labelled material has demonstrated that peritoneal mast cells display different surface proteins than lymph node, spleen or thymus cells. The dominant protein peak, with a molecular weight of

about 30,000, has been found to be a unique mast cell protein. The major protein dissociated from Hooded Lister mast cells by low pH treatment is IgE. Others attempted to identify an IgE receptor (Conrad et al., 1976; Conrad and Froese, 1976; Kulczycki et al., 1976). Some of these experiments have been repeated and the results indicate that the molecular weight of the receptor can be about 60,000 or multiples of that unit.

Although it is well documented that the IgE-mast cell system plays an important role in allergy, the question of IgE level differences between animal strains has not been previously addressed as a sufficiently sensitive IgE assay has not been available. The results of hybridization experiments indicate that the IgE level must be regulated by more than one gene. High IgE level is not dominant in combination with low IgE level. It would rather appear as though low level strains have a dominating suppressor function. The high IgE level strains could be "defective" in the regulatory mechanisms in the same way as allergics, i.e. they express high total IgE levels and also respond with a high amount of IgE antibody upon sensitization. A basic potential for IgE production exists even in low IgE level rats and these rats possess all the other mechanisms in the IgE-mast cell system. It appears probable that a considerable amount of information can be obtained from the rat model system if sensitive IgE assays are used and if the IgE responses are studied in concert with antibody responses of all other immunoglobulin classes. Perhaps the strain differences in IgE levels might lead to the formulation of new theories and systems to study regulation of antibody synthesis.

VI. SUMMARY

This thesis presents a new system for the quantitative measurement of total IgE (PRIST) and IgE antibody (PRAST) in rat serum. The radioimmunoassays are based on paper disc coupled anti-IgE antibodies and antigen, with ^{125}I anti-Fc-ε used as an indicator of IgE uptake, and allow the measurement of total IgE and IgE antibody below 1 ng per ml and are thus more sensitive than other rat IgE assays described to date. PRAST is the first in vitro assay that has been used for rat IgE antibody. PRAST was also adopted for evaluation of the relative amount of IgG antibody produced in immunization experiments. Differences between rat strains in normal serum IgE levels and in IgE antibody production after parasitic infection and immunization are shown. Hybridization experiments indicate a poly-gene control of the serum IgE level. Experiments with isolated rat mast cells show differences between a high IgE strain (Hooded Lister) and a low IgE strain (Sprague Dawley) in the number of occupied IgE receptors. The number of IgE receptors per mast cell is independent of the serum IgE level of the strain. Histamine release experiments with isolated mast cells, nonsensitized and passively sensitized, show a better immune reactivity of cells from the high IgE than the low IgE rat strain. Overly high serum IgE levels are shown to interfere with the use of these rats for IgE antibody determination by passive cutaneous anaphylaxis. An attempt to quantitate the amount of IgE antibody necessary to give a positive PCA response is also made. External labelling of cells from the immune system show unique proteins on the surface of mast cells and other peritoneal cells.

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